Growth variation of wild and cultured populations of the European eel *Anguilla anguilla*, L.

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I hereby declare that this thesis is the result of
my own work except where stated otherwise.

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ABSTRACT

The growth variation of the European eel, *Anguilla anguilla* L., was investigated in both wild and cultured populations. An ecological study was conducted in the River Almond, Midlothian, Scotland and five growth experiments were performed in a warm water recirculation system.

In the River Almond, growth variation was apparent in glass eels once they joined the benthos and commenced feeding, and was pronounced in the eel population, where there was a wide size range in each age class with considerable overlap in adjacent age classes. Growth rate affected body composition, and lower nutritional condition was demonstrated by lower lipid levels of slow growing eels, and those having high length to weight ratios, in the wild and in culture. It was suggested that these individuals in the wild would have less chance of reaching maturity before they died. Water temperature was considered to be the major factor limiting growth and eel feeding activity increased above 10 °C. Growth rate may have been reduced by population density which was higher below a weir, which acted as a partial barrier to upriver migration of glass eels and young eels.

Growth variation was more extreme in culture than in the wild, which was related to increased water temperature and greater access to food, which in turn increased growth rate and agonistic behaviour which induced growth depensation. The physical properties of tubifex significantly affected growth, which was highest on the diets which most closely resembled natural food. Survival was 100 % and instantaneous growth rate over 3.5 % day^{-1}, when agonistic behaviour was reduced to a few minutes at feeding time, but variation in growth continued and was considered to have some genetic origin. Stress was induced by feeding dry diet and by agonistic behaviour but the stress response was reversible. Hierarchical position was determined largely by size but may have been influenced by prior social experience or genetic factors. That population growth in culture may rely, to some extent, on the development of dominant fish at the expense of subordinates was suggested. In conclusion, the experiments have shown that further knowledge of eel biology could be used to improve the efficiency of utilization of the eel resource, both through fishery and culture management.

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CHAPTER 1

INTRODUCTION AND GENERAL METHODS

1.1 INTRODUCTION

Growth variation in wild and cultured European eel *Anguilla anguilla* is pronounced. In the wild there is a wide size range in each age class with considerable overlap in adjacent age classes (Frost, 1945; Deelder, 1957; Sinha and Jones, 1967a). For example, in the Welsh River Ffraw eels of 20 cm were between one and three years old (Sinha and Jones, 1967a). In high density culture in warm water, some individuals grow particularly well whilst up to one third of the stock may show no growth at all and may have to be discarded (Wickins, 1983).

Variation in growth occurs between the sexes and between and within individuals, and can be divided into environmental and genetic components. The growth of the different sexes is an unknown quantity and might play a substantial role in growth variation and competitive behaviour. The sex determination mechanism is not fully understood but there is evidence to suggest that sex is genotypically determined, where the female is regarded as the heterogametic sex, and the chromosome mechanism is assumed to be of the ZW/ZZ type (Passakas, 1976). However, differentiation of the gonads is labile and can be influenced by environmental factors such as temperature, stocking density and food quality and quantity (Kuhlmann, 1974). There are no external morphological sex-linked characteristics to distinguish between the sexes until their metamorphosis into 'silver' eels and migration to the spawning grounds. Karyological techniques are being developed with *Anguilla anguilla* but are producing contradictory findings and are restricted to larger eels (Beeckman and Ollevier, 1987). Histological techniques are possible once the gonads have differentiated, which is usually between 20-30 cm but is not necessarily related to age (Sinha and Jones, 1966).

Individual growth variation can arise through environmental and genetic variance. Variation over time within individuals must be due to environmental effects as the genotype remains constant. Variation between individuals will be due to any environmental effects, for example, those contributing to a stable hierarchy, together with genetic variance (Purdom, 1974).

The extreme growth variation between eels in the wild and in culture has considerable bearing on management in fisheries and aquaculture. Methods of stock assessment in the natural fishery are complicated by having a wide size range of fish within a single year class, and sexually mature individuals which emigrate from the fishery at different ages. Management is confounded further by the eels life cycle, geographical range and migratory habit. The catadromous life cycle involves a migration of silver eels to spawning grounds in the Sargasso Sea (26 °N 56 °W) and a return migration of 'glass eels' to continental coastal and freshwaters, where growth and development into 'elver' and then 'yellow eel' proceeds (Schmidt, 1912). The geographical distribution of the species is extensive, that is, north-south from Iceland to the Canary Islands and east-west from the Black Sea to the Bermuda Islands. The growth phase in continental waters often involves considerable migration through river systems, and distances travelled can be extensive, for example, 20 km per year in the River Severn, England (Aprahamian, 1988).

In culture losses due to growth variation are related to the effect that agonistic behaviour has on appetite and feeding. Growth inequality is maintained by development of a hierarchy, where more activity is forced on subordinates which receive less food and are subject to more stress, which reduces appetite and resistance to disease. Despite frequent size-grading, cannibalism, mortality or stunting can result and populations of fish of mixed size can lead to inappropriate food sizes, waterflow volumes and stocking densities.

Eels do not breed successfully in captivity and eel culturists are dependent upon a natural source of stock which becomes available as part of an annual migration of glass eels into coastal and inland waters. There is room for improvement in methods of capture, transportation and transfer to culture. Capture methods are often wasteful of resource and effort and need to be optimized and standardized to allow comparison of catch statistics with time and through geographical range (Dekker, 1987). Transportation presents a high disease risk where fish and water are moved across the world, as demonstrated by the introduction of the swimbladder nematodes *Anguillicola* spp. into the European eel from South East Asia (Sarti *et al*, 1985; Egusa, 1979). Transfer to culture can involve acclimation to water of differing temperature and salinity before first feeding and weaning on to a dry artificial diet. This is a critical time when fish are particularly susceptible to stress and disease and variation in growth can be extreme, even in elvers adapted to dry food (Kuhlmann and Koops, 1980).

In this study the growth of a wild population of eels in the River Almond, Scotland was examined with the aim of gaining a better understanding of the ecological

significance of extreme growth variation. It was hoped that an understanding of the interaction and interrelation of various environmental factors would help to standardize similar environmental variables in the culture system and interpret results of a series of growth experiments, which were designed to examine the effect of culture conditions on growth variation.

The growth of the wild eel population is described in the first part of the thesis which is followed by consideration of the effects of transfer of glass eels to culture and finally to the effects of culture conditions on growth variation. Chapter 2 is divided into five sub-sections which examine the effect of various factors on the diet of eels in the River Almond, which include the effects of seasonal and hydrological factors on food availability and feeding intensity, and the effects of food availability, eel morphology and feeding preferences on food selection. Chapter 3 is concerned with age determination of eels and the intrinsic factors affecting growth including age, sexual maturity, sex and heredity. Other more extrinsic factors affecting growth including parasitism, disease, competition, predation, human intervention and other environmental factors are discussed in Chapter 4. In Chapter 5 the effect of growth rate, weight to length ratio, sexual differentiation and seasonality on the body composition of wild eels is examined. In Chapter 6 the glass eel migration and development during the first year in freshwater in the River Almond is described. In Chapter 7 the effect of an extended holding period under natural conditions, and first feeding and weaning on growth was examined. Two experiments are described in Chapter 8 which were designed to examine the influence of diet and social interaction on growth variation in culture, with reference to the degree that each factor in culture departs from the situation in the wild. In Chapter 9 the effect of stress induced by type of diet and social interaction was examined in two experiments.

Both eel fishery and culture industries are dependent upon a natural recruitment of glass eels. Some water catchments support three anguillid fisheries, for example, glass eel, yellow and silver eel in the Severn catchment, which can place severe pressure on the resource. Eel production was found to be directly correlated with standing crop in the Severn (Aprahamian, 1986) and the fact that glass eel catches have been declining during the period 1981 to 1986 (Moriarty, 1987a) indicates the need for effective management of the resource and further research to ensure the continuation of the industries and maintenance of the species. This thesis is an attempt to contribute to the understanding of growth variation of *Anguilla anguilla* and to evaluate its effect in warmwater high density culture.

1.2 GENERAL METHODS

1.2.1 NATURAL FEEDING ECOLOGY AND GROWTH

1.2.1.1 STUDY AREA

Catchment

The River Almond rises among the Cant Hills, at an altitude of 274 m, approximately 5 km south-west of Harthill, Lanarkshire, Scotland (Figure 1.1). The river flows for 50 km in a N.E. direction until it reaches the Forth Estuary at Cramond, Edinburgh, where it discharges onto the inter-tidal Drum Sands. The River Almond drains a catchment area of 375 km^2 which includes sewage, ferruginous, industrial and agricultural effluents. In the upper reaches the river flows through poorly drained and unimproved acid grassland, which is utilized for cattle and sheep grazing, and areas of coal measures formed from Carboniferous drifts of sandstones, shales and fireclays. As the river progresses towards the coast the soil becomes increasingly fertile with brown forest soils with gleying followed by brown forest soils, derived from rocks of the Carboniferous Limestone Series often interspersed with millstone grit and outcrops of igneous rocks. There are also localized pockets of recently deposited alluvial soils. The land is utilized predominantly for grassland with some coniferous plantations in the middle reaches, becoming increasing arable in the lower reaches with dense deciduous woods flanking the river at Cramond. Water is polluted from disused quarries, an ironstone bing, which is buried in an opencast coal site, run-off of fertilizers from free-draining arable land, and silage and slurry from piggeries. In addition, there are five sewage works and effluents from a papermill and breweries, where the human population is concentrated along the river in the middle and lower reaches.

Climate

The climate is temperate, and the upper reaches are defined as fairly warm and moderately dry becoming warm and moderately dry in the lower reaches (Brown and Shipley, 1982). Moist westerly winds rising over the Southern Highlands lead to greater precipitation on the higher ground in the west, near the source of the River Almond, giving an average annual rainfall of 1026 mm at 241 m, whilst on the lowlands at Cramond, at 61 m, the annual average rainfall is 715 mm.

FIGURE 1.1 Location of the River Almond, Scotland
(Scale 1 : 4,000,000)

Sampling sites

The sampling sites were situated in the lower reaches of the River Almond above and below Cramond weir, the latter site being immediately above the furthest point of tidal influence. The river bed was approximately 25 m wide and the water slow moving, along a gradient of less than 0.25 %.

Site 1 (NT 189767) (Figure 1.2) below Cramond weir, was 1 km from the river mouth. The river bed consisted of large boulders, rocks and gravel with some stone spoil which had been dumped in the river. There was quite dense growth of filamentous algae *Cladophora glomerata* attached to the rocky substrate. There were muddy shallows near the river bank and sewage fungus *Leptomitus lacteus* and associated organisms (Curtis and Curds, 1971) present during the summer.

Site 2 (NT 182761) (Figure 1.3) was 0.5 km above Cramond weir. The river bed consisted of large boulders, rocks and gravel and there were muddy areas in shallows near the river bank. Filamentous algae was present but sewage fungus was absent.

Site 3 (NT 188766) (Figure 1.4). The area around the fish ladder in Cramond weir. The weir, a listed monument, is 25 m wide with a 3 m fall.

FIGURE 1.2 Site 1 (NT 189767) The River Almond below Cramond weir

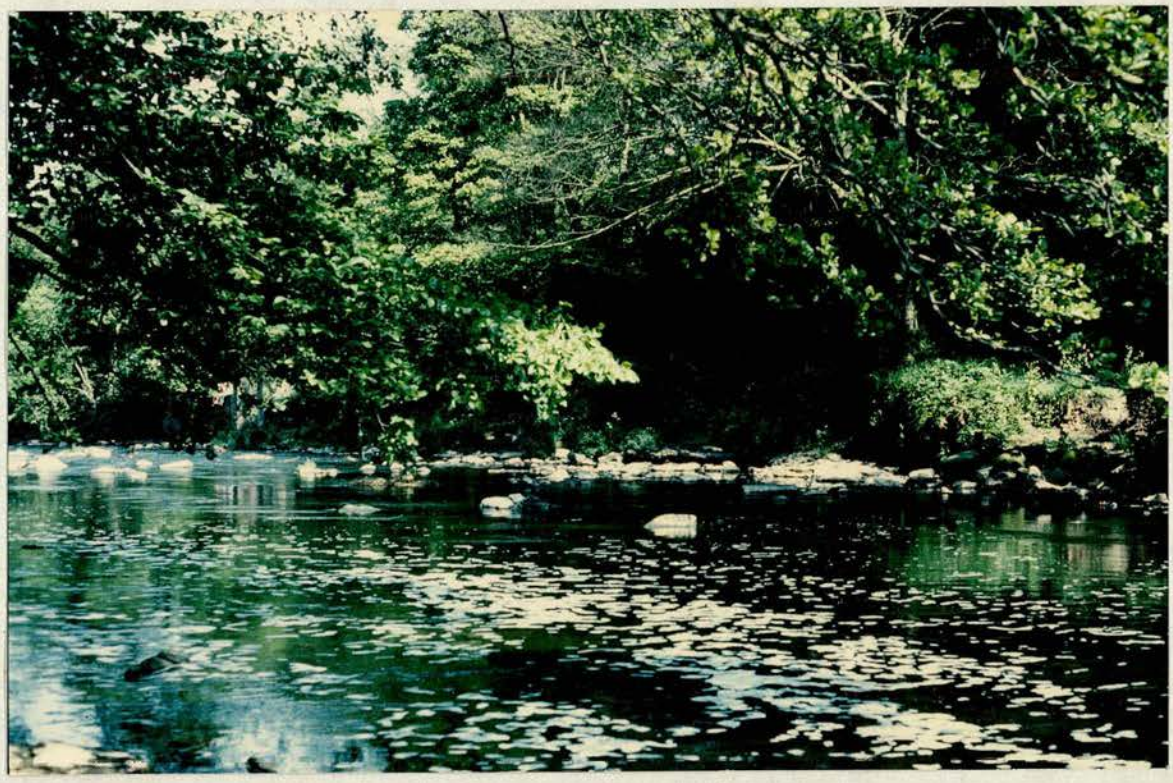


FIGURE 1.3 Site 2 (NT 182761) The River Almond above Cramond weir

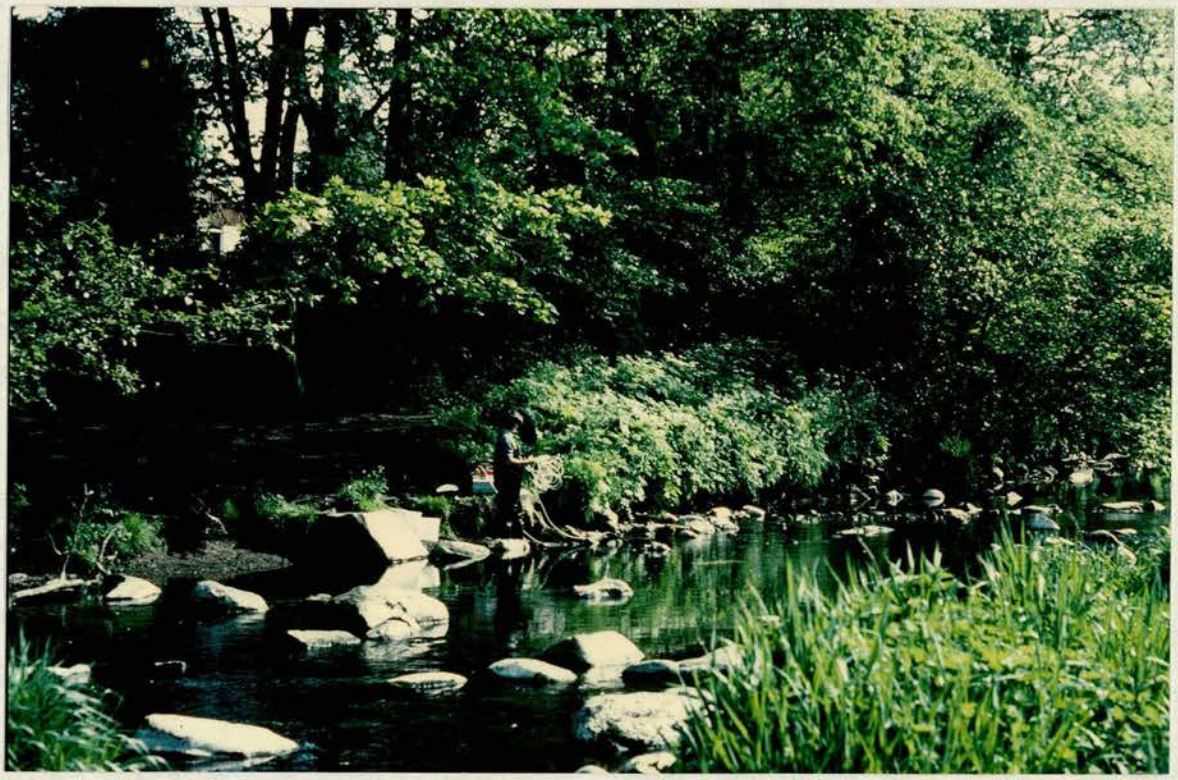


FIGURE 1.4 Site 3 (NT 188766) Cramond weir and fish ladder

1.2.1.2 SAMPLING PROCEDURE

The growth of eels in the River Almond was determined by sampling the population at Site 1 and Site 2 at monthly intervals, over a 13 month period, from June 1985 to June 1986. The feeding activity of the eel is mainly nocturnal (Sinha and Jones, 1975) and samples were collected in the early morning to minimize the effects of digestion. At the same time, food availability was assessed by sampling the bottom fauna and the abiotic conditions were recorded. On separate occasions samples of glass eels were taken during their migration, and at the weir (Site 3), as described in Chapters 6 and 7.

Fishing method

The eels were caught by electrofishing which was chosen as the most suitable fishing method. A wide size range of eels is sensitive to the electric field (Boccardy and Copper, 1963) which induces galvonarcosis (Halsband, 1967). This impairs swimming and balance and may cause the fish to roll over, which induces a relaxed condition where they remain easy to catch in hand nets until the current is switched off. The fishing method is effective regardless of the nature of the habitat and is useful in drawing fish from cover provided by undercut banks, overhanging vegetation and a rocky bottom. The method is also suitable for collection of stomach contents for analysis of food items because regurgitation of food, digestion after capture and the risk of catching fish in abnormal situations are minimized (Windell and Bowen, 1978). The fish were transferred from hand nets to buckets filled with water. They were killed with an overdose of benzocaine anaesthetic (Ethyl - 4 - aminobenzoate dissolved in ethanol at 100 g. l^{-1}).

The electrofishing device consisted of a pulsed direct current power supply consisting of a 500 watt 'Honda' generator and transformer (Delta mark VII control unit, Aquatic Services (International) Ltd., Heath Lane, East Boldrie, Hampshire, England.) which powered a pair of electrodes, a portable anode and a steel mesh framed cathode.

The same area (100 $m^{2)}$ at each site was electrofished each month. It was not practicable to use stop nets to enclose the sample area because of the extreme width of the river (25 m) which was strewn with bolders, and was often fast flowing and deep towards the middle. The sample area followed 20 m of river bank and 5 m into the river, which was near wader height on occasions. Therefore, the sample numbers do not reflect the true population density but are an indication of lowest density, as there was thought to be little immigration into the sample area during fishing.

Analysis of fish

Measurement of weight and morphometric characters were taken before freeze storage. The gut contents can increase the weight by 100 % (Tesch, 1973) and thus weight minus the gut contents was recorded, on a Sauter electronic balance to the nearest 0.01 g, for eels 1 g and over, and 0.001 g for eels less than 1 g. Total length (from the tip of the snout to the tip of the caudal fin) was measured with a V-shaped measuring board, or ruler for the smaller eels, to the nearest 0.1 cm. Interpupillary distance and head length (from the tip of the snout to the insertion of the pectoral fin) were measured with vernier calipers to the nearest 0.01 cm.

Sex was determined by macroscopic examination of the gonads. The ovaries are frilled and ribbon-like organs (Bertin, 1956) and in the eels examined were pink and often transparent and quite delicate. The testes were white, lobed organs, which were quite solid structures running down the length of the body. It can be difficult to distinguish between testes and the lobulate organ, which may develop into either ovaries or testes (Sinha and Jones, 1967a), although it was not a problem in this study where only two males were caught. Eels without recognizable gonads were classed as sexually undifferentiated or immature.

Age was determined by examination of the otoliths (Chapter 3) and body composition was determined by proximate analysis (Chapter 5). Feeding activity was assessed using an index of stomach fullness (Chapter 2.4.1) and the nature of the diet was investigated by analysis of the stomach contents (Chapter 2.3.1).

Benthic fauna

The habitat at Site 1 and 2 was sub-divided into substrate types that were predominantly rocky or muddy. Kick-sampling was used (Macan, 1958) and two samples from each habitat type were taken at each site (Chapter 2.3.1).

Abiotic conditions

The abiotic conditions recorded included water temperature, weather conditions and the flood and pollution status of the river (Chapter 2.2.1).

1.2.2 GROWTH EXPERIMENTS

Intensive eel culture in temperate regions requires water temperatures to be elevated considerably above ambient and 26.5 °C is considered optimum (Kuhlmann, 1974). Warm water discharges from industrial processes (Sadler, 1979), geothermally heated water and closed water recirculation systems can provide such conditions reasonably economically. A closed recirculation system was used in this study which consisted of 28 experimental tanks in parallel, 2 header tanks and 5 filter tanks. The filters removed both solid wastes, that is, excess food and excreta, and ammonia, the main nitrogenous waste product of fish. Dissolved oxygen was depleted, through bacterial breakdown of solids and nitrification of ammonia and from the respiratory demands of the fish, and was replaced by aeration. A **pilot trial** was conducted to test the experimental system, maintenance procedure and analytical methods. The problems that arose, and changes that were made, are incorporated in brackets in the description of the experimental methods. Methods specific to a particular experiment are described in the relevant chapter.

1.2.2.1 RECIRCULATION SYSTEM (FIGURE 1.5)

Water supply

A mains freshwater supply was used for the closed recirculation system (there was mortality of fish during the pilot trial due to chlorine in the water and the problem was solved by maintaining vigorous aeration in the header tanks). Temperature was elevated to 25 °C and maintained to within ± 1°C with a thermostat and 1 kilowatt heating element. Initially this was positioned in the upper header tank in the pilot trial but was moved to the sump because turbulence, due to the vigorous aeration, was liable to dislodge the heater.

The recirculation system consisted of two elevated header tanks, the upper was connected to the mains water supply and automatically supplied the lower system header tank through a standpipe. There was a standpipe in the system header and the overflow was directed into the sump. The water supply to each header tank was controlled with a ballcock. Water flowed through the experimental tanks from the system header tank and was controlled with valves at each inlet. Water drained through a central 'monk' outlet from each experimental tank to a central gutter which took the water to four settling tanks in series. The water was directed to the bottom of each sedimentation filter tank via a down-pipe, and trickled through a gravel biological filter (biofilter) positioned above the sump, from which water was pumped to the system header tank.

Water replacement.

The total water capacity of the system, that is, 0.89 m^3, was pumped around more than once an hour by water flowing through the system at 1.5 m^3 per hour. Water volume was kept constant with an automatic supply of freshwater which replaced losses due to evaporation and siphoning waste. Complete turnover of water was estimated to take one month.

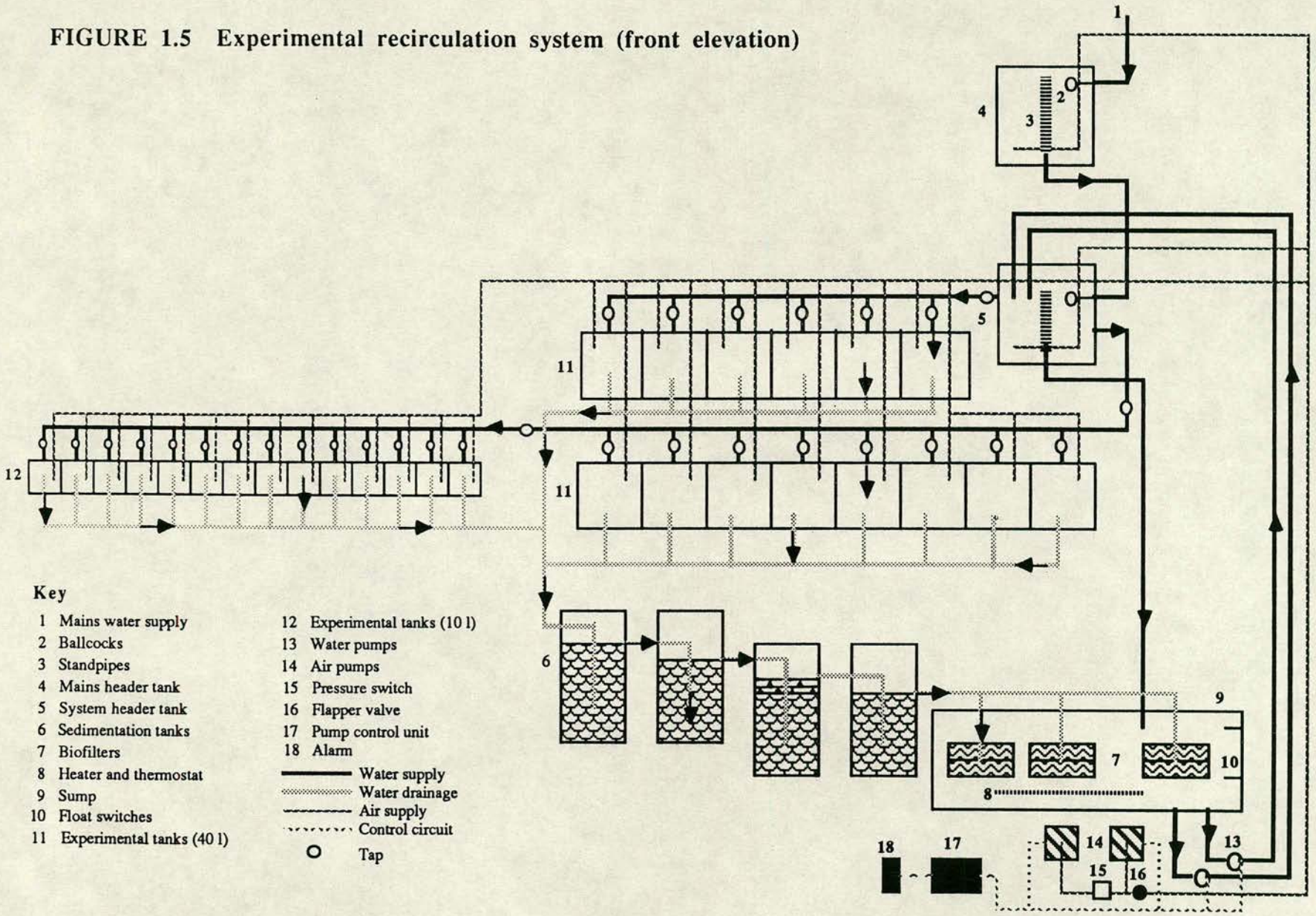
Water quality

The sampling methods used and the mean values of the water quality factors measured are shown in Table 1.1. Values of temperature, dissolved oxygen and pH were recorded daily with a probe placed directly into an experimental tank and water samples for analysis of ammonia and nitrate levels were taken twice a week. The water quality was tested approximately four hours after feeding, when the demands on the system were expected to be high (Poxton and Allouse, 1987).

TABLE 1.1 Water quality analysis

Factor	Mean	±	Method
Temperature °C	25	1	'Phox 62' meter
Dissolved oxygen mg. l^{-1}	8.0	2.0	'Phox 62' meter
pH	7.0	0.5	'Gallencamp' dipstick
Nitrate mg. l^{-1}	3.0	2.1	'Tecator' flow injection auto-analyser
Nitrite mg. l^{-1}	0.01	0.005	'Tecator' flow injection auto-analyser
Ammonia (NH_4-N) mg. l^{-1}	0.7	0.7	'Tecator' flow injection auto-analyser
Ammonia (NH_3-N) mg. l^{-1}	0.004	0.003	Calibration curve (Trussel, 1972)

FIGURE 1.5 Experimental recirculation system (front elevation)

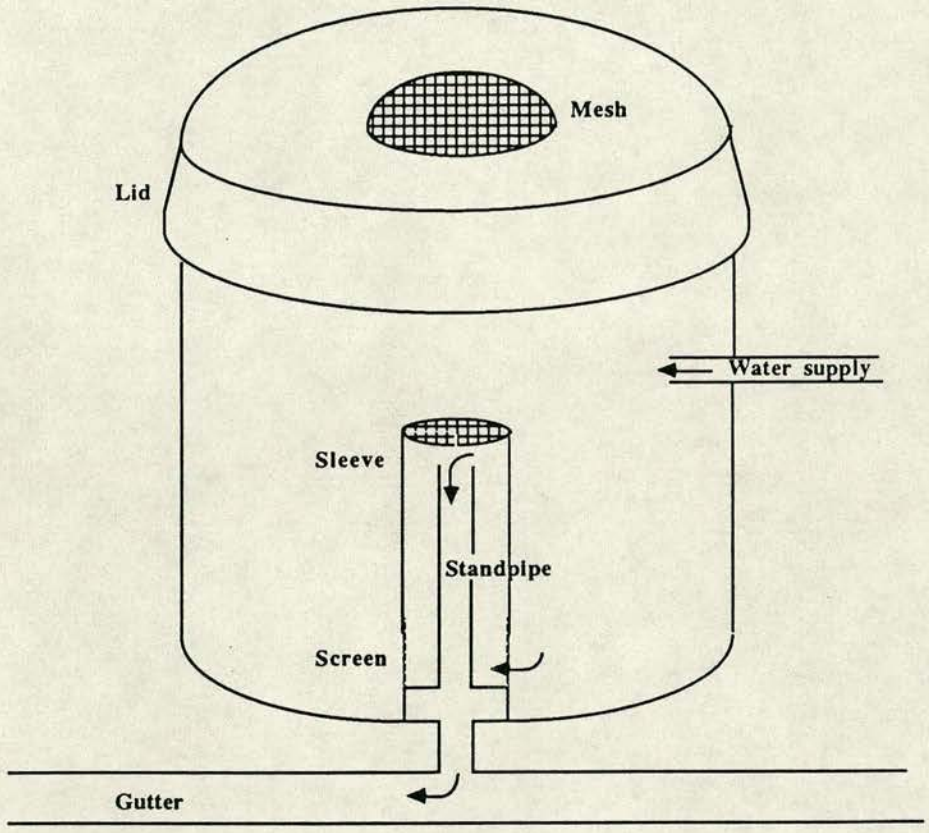


Key

1 Mains water supply
2 Ballcocks
3 Standpipes
4 Mains header tank
5 System header tank
6 Sedimentation tanks
7 Biofilters
8 Heater and thermostat
9 Sump
10 Float switches
11 Experimental tanks (40 l)
12 Experimental tanks (10 l)
13 Water pumps
14 Air pumps
15 Pressure switch
16 Flapper valve
17 Pump control unit
18 Alarm

Water supply
Water drainage
Air supply
Control circuit
Tap

FIGURE 1.6 Experimental tank



A high standard of water quality was maintained by mechanical and biological filtration, pH buffering, aeration and water replacement. Also, large solid wastes were siphoned from the tanks daily. Filtration to reduce degradable solids, and prevent ammonia build-up was considered to be sufficient, and further treatment with activated carbon and ion-exchange filter systems deemed unnecessary, because of the relatively short holding period of a few months (Muir, 1976).

Excess feed and wastes were removed by sedimentation from water flowing upwards through randomly arranged plastic media filter material of 7 cm diameter. Solids were assisted in clearing downwards by gravity and hence the passage through the filter were not blocked and no backwashing was necessary. The filter tanks were arranged in a series which allowed their removal for washing without interruption of the flow of water to the biofilter. The sedimentation filters were washed once every two weeks in a series of one tank per day, to avoid temperature or water quality shock to the system with the large input of top-up water. Water was sprayed downwards onto the biofilter, introducing additional oxygen which is required for bacterial activity, onto a mixture of Dorset pea gravel and a calcium/magnesium based gravel 'Calcium plus' (Underworld Products Ltd.) of 0.5 - 1.5 cm particle size, in the ratio of 5 Dorset pea gravel to 1 'Calcium plus'. The gravel mixture made a layer 3 cm deep in 6 trays, arranged in 2 vertical rows, which were positioned in the sump. The population of nitrifying bacteria (*Nitrosomonas* spp. and *Nitrobacter* spp.) was built up by preconditioning the system before the experiments started. An indication of the biological capacity can be gained by the following mean values:

	before filter	after filter
$NO_3 - N$ mg. l^{-1}	0.7	5.00
$NH_4 - N$ mg. l^{-1}	1.5	0.13

The oxygen level was maintained by pumping air into the header tanks and into each experimental tank. Both air and water pumps operated with a back-up pump, which were activated automatically by a pressure switch and two float switches respectively. The two float switches were positioned to control the highest and lowest water level (Figure 1.5). An alarm was triggered if a back-up pump was activated which enabled a fault to be identified and rectified rapidly.

Lighting

The lighting was subdued by a red filter and on a constant illumination period from 08.00 h to 18.00 h. The eels were less nervous in subdued light and yet they

remained clearly visible for observation in the red light. A light periodicity was maintained that approximated conditions in the wild.

Experimental tanks

The experimental tanks were arranged in parallel, in two blocks, one of 14 x 40 l and one of 14 x 10 l volume, where a standpipe in the middle of the tank maintained the water volumes at 20 l and 5 l respectively. The circular tanks were designed to be self-cleaning (Figure 1.6). However, solids trapped by the mesh screened outlet, and not removed by lifting the outlet standpipe and discharging into the gutter, were siphoned out daily. At each weighing period, after removal of fish, the water was returned to the recirculation system via the sump and the tanks were cleaned before refilling with water from the header tank and replacing fish. The eels were prevented from escaping with mesh screens at water inlets and outlets, and with a lid on each tank (a hole was cut in the plastic tank lid, which was covered with mesh to allow better circulation of air in the system, which had become fetid during the pilot trial). There was no shelter provided for the eels during the growth experiments, except for gravel in particular experiments where the effect of substrate was examined.

1.2.2.2 MAINTENANCE PROCEDURE

Origin of the stock

Elvers were obtained from both the River Almond, Scotland and a commercial source (Bristol Channel Fisheries Ltd., 123 Hempsted Lane, Gloucester, England). Eels were kept in holding tanks which were isolated from the experimental system in periods between experiments and for quarantine purposes. These operated on a flow-through system of water at ambient temperature which ranged from 8 to 18 °C. The eels were fed live tubifex, as a first feeding diet, and during weaning on to a commercial dry diet. Dry food was dispensed from automatic feeders over a 24 hour period. The tanks were cleaned and dead fish removed daily.

Transfer

Fish were graded individually by weight, to reduce the initial size variation and subsequent growth variation (Jobling and Reinsnes, 1986), before transfer to an intermediate tank, where the holding tank water was replaced by that in the recirculation system over a period of 24 hours. Fish were not fed during this time.

Disease treatment

To prevent the introduction of ectoparasites the eels were bathed, on arrival, in a treatment of formalin and malachite green, applied at a concentration of 1 mg. l^{-1} malachite green and 167 mg. l^{-1} formalin for one hour. Treatment was not successful in preventing the introduction of *Ichthyophthirius multifiliis* (white spot) into the

recirculation system. This ciliate protozoan is extremely difficult to treat both in its trophant stage within the epidermis of the fish and when encysted, when it can lie dormant on the bottom of the tanks (Poupard, 1978). Whitespot was controlled with a commercial preparation of malachite green, acriflavine and quinine sulphate (W.S.3 King British Aquarium Accessories Co. Ltd., Bradford, Yorkshire, England) which was administered as a prophylactic treatment into the water in the recirculation system without affecting the activity of the biological filter.

Feeding

Tubifex and dry food in crumb and paste form were used in experimental diets. Tubifex is a common first feeding diet in the wild (Table 2.8) and culture (Kuhlmann, 1974). All worms belonging to the family Tubificidae are commonly referred to as 'tubifex worms' as samples are often made up of more than two genera, that is, *Tubifex* and *Limnodrilus*. The eels were fed on the basis of a percentage of body weight per day equivalent to 5 % dry weight, which was adjusted after each weighing interval. The ration was broadcast evenly amongst the fish to help subordinates obtain food (Jobling, 1983; Wickins, 1983), twice a day at 0900 h and 1700 h. Bulk weights of tank populations were not used for ration adjustment because there was greater variation in the weight obtained compared with the total of individual measurements. This may have been due to water retained over the large surface area of the eels.

Live tubifex were obtained from commercial sources and were treated with a proflavine (3, 6-Diaminoacridine, Sigma Chemical Company) bath at a concentration of 1 g. l^{-1} for one hour to prevent the introduction of bacteria and protozoa into the recirculation system. The tubifex stock was maintained in shallow trays with a supply of running water and was cleaned daily by disturbing the clump it naturally forms, to remove wastes collected within and under it. The stock was renewed once a week.

Two types of commercial dry food were used, that is, 'Ewos Baker with Norsabel' (Ewos Baker Ltd., Bathgate, West Lothian, Scotland) and BP Mainstream feed for eels (B.P. Nutrition (UK) Ltd., Preston, Lancashire, England). 'Ewos Baker with Norsabel', a diet formulated for salmonids, was recommended for use by eel farmers before a specialist eel food was produced in the UK. The proximate analysis and particle sizes of the diets are shown in Table 1.2. The particle sizes are within the range for eels weighing up to 0.2 g (0.43 - 0.85 mm) and 0.2 - 5.0g (0.80 - 1.40 mm) (Knights, 1983).

TABLE 1.2 Proximate analysis and particle size of commercial eel diets (NFE=nitrogen-free extractives)

	'Ewos Baker with Norsabel'		'BP Mainstream'	
Proximate analysis				
Moisture	6.48		8.22	
Protein	55.73		51.74	
Lipid	18.76		11.01	
Ash	11.04		10.66	
NFE	7.99		18.37	
Particle size	0	0.3 - 0.6 mm	00	0.2 - 0.8mm
	1	0.6 - 1.1 mm	01	0.5 - 1.4mm
	2	1.1 - 1.5 mm	02	1.0 - 2.0mm

1.2.2.3 ANALYTICAL METHODS

Growth measurement

Eels were starved for one day before measurement of growth to prevent distortion of weight because of stomach contents. Eels were removed from the experimental tanks and anaesthetized in MS 222 (Tricaine methane sulphonate) Sandoz solution (Sandoz Ltd., Basle, Switzerland) at a concentration of 1 part per 3,500 water (from the recirculation system) over a 2 to 5 minute period. The effect on muscular activity is rapid. (MS 222 was easier to use than benzocaine, which formed a white precipitate, and the additional cost was offset by the small quantities required). Excess water was removed by blotting on paper towels before measurement of length to the nearest 0.1 cm, with a ruler, and weight to the nearest 0.001 g, on a Sauter RE1614 electronic balance. Traces of anaesthetic were rinsed from the fish before returning to the recirculation system where their recovery was almost immediate.

Losses

Losses occurred during the experiments due to mortality or escape. Mortality was caused by infection with *Ichthyophthirius multifiliis* (whitespot) or due to damage sustained during aggressive chase and bite behaviour which affected osmotic balance. No cannibalism was observed. Dead fish were removed and length and weight recorded. Losses were made up before the first measurement interval in *Experiment*

8.1 only. Dead fish tended to be the smaller individuals (Figure 7.2), with the result that population mean and median weights were increased, however no correction was made (Kuhlmann, 1974). There was no adjustment of ration due to mortality until the end of each measurement interval. Losses were expressed in terms of percentages of survival or mortality during each measurement interval or over the whole experimental period.

1.2.2.4 GROWTH ASSESSMENT

Significance of the differences in growth between the treatments

Growth variation between individuals within populations of *Anguilla anguilla* increases with time, due to growth depensation, where the large fish grow even larger and the smaller fish lag further behind. An original normal distribution of glass eels or graded eels spreads at both ends of the scale, that is, at the lower end by eels who do not feed at all and lose weight, and by a relatively small proportion, approximately 5 - 10 % (Wickins, 1983) of fast growers who deflect the distribution to the right, giving the population distribution a positive skew. The effect of the fast growers on normal population statistics is large, whereas the small number of extremely slow growers is not so important (Kuhlmann, 1974). It is not appropriate to use normal theory (parametric) statistics to describe the population growth of *Anguilla anguilla* where results could be misleading. Distribution-free (non-parametic) methods are more efficient than normal theory procedure when sampling from a population that is not normally distributed, while methods using the median rather than the mean are more resistant to distortion by occasional outliers. The Mann-Whitney *U*-test is used to test whether two independent groups have been drawn from the same population. It has a power efficiency of 95.5 % when compared to the *t* - test, the most useful parametric alternative (Siegel, 1956). When three or more samples were compared an over-all difference among the samples was tested by a Kruskal - Wallis procedure, which has a power efficiency of 95.5 % when compared with the F - test. Statistical tests were performed using the computer package Minitab, (Minitab, Inc., Pennsylvania State University).

Rate of growth (G)

The logistic growth curve (D'Arcy Thompson, 1942) is used mainly to describe the increase in weight of fish populations rather than individuals (Schaefer, 1954 in Ricker, 1979). Instantaneous rate of increase in weight (G) (or specific growth rate) is determined thus,

$$G = (\log_e W_2 - \log_e W_1 / T_2 - T_1) \times 100$$

where W2 is the median weight at time T2 and W1 is the median weight at time T1,

with weight expressed in grams and time in days. It is equivalent to the % increase in weight per day and in this thesis is used as 'rate of growth' involving change in weight irrespective of length.

Growth variation

The relative amounts of variation between populations is assessed by determination of the coefficient of variation (CV), that is, **CV = 100 x standard deviation / mean**. CV can be used to study changes that occur within a population and to compare different populations or populations of different fish (Purdom, 1974). An increase in CV indicates divergence in growth which can be due to growth depensation and the formation of a social hierarchy (Brett, 1979) or can be independent of social interaction. Small individuals may be more sensitive to changes in environmental conditions, such as dissolved oxygen levels and temperature, which could lead to differential physiological stress and suppression of growth independent of social interaction (Jobling, 1985). If there is no change in CV with time it signifies either that interaction is not influencing growth, or that behavioural interrelations are completely random.

CHAPTER 2

FEEDING

2.1 INTRODUCTION

Eels display great trophic opportunism in their diet. The spectrum of food consumed ranges from fish, through Mollusca, Crustacea and Insecta to annelid and platyhelminthe worms. Plant material and detritus are also found in the gut contents but are considered to have been ingested unintentionally whilst taking animal prey (Tesch, 1973).

This chapter is divided into five sub-sections which examine the effect of various factors on the diet of eels in the River Almond, Scotland. These include the effects of seasonal and hydrological factors on food availability and feeding intensity, and the effects of food availability, eel morphology and feeding preferences on food selection. The discrimination shown by the eel in its choice of food items, and whether this involves selection of individual organisms, or gathering like a detrital feeder, is considered.

Food availability was assessed through sampling of the bottom fauna, as eels derive most of their food from the zoobenthos (Tesch, 1973). The nature of the diet was assessed by analysis of the stomach contents, a standard practice in fisheries ecology (Hyslop, 1980). Samples of the benthic fauna and the eel population were made, and the abiotic conditions recorded, at monthly intervals over a period of thirteen months, from June 1985 to June 1986, at two sites on the River Almond. (The general sampling procedure and sampling methods are described in Chapter 1.2.1).

2.2 ABIOTIC FACTORS

The distribution and abundance of animals and the productivity of the water body are influenced by various abiotic factors including seasonality, the nature of the substrate, action of the water current, local geology, land use and discharge of pollutants.

2.2.1 METHODS: The abiotic conditions recorded at the time of sampling included water temperature, weather conditions and the flood and pollution status of the river. The presence of algal growth *Cladophora glomerata* and sewage fungus *Leptomitus lacteus* plus associated organisms, which can include bacteria, other species of fungi, algae and protozoa (Curtis and Curds, 1971) was noted. The nature of the substrate was taken into account by sub-sampling in rocky and muddy habitats. Reference was made to Forth River Purification Board water quality data, that is, from monthly sampling at a point less than one kilometre upstream at Cramond Bridge (NT 180755) and continuous recording water temperature and flow data at Cragiehall (NT 165752).

2.2.2 RESULTS AND DISCUSSION: The abiotic conditions at the times of sampling were similar at both sites and are shown as combined mean values in Table 2.1, where the mean daily flow rate between the sampling times is included. Monthly mean values for water quality parameters are shown in Appendix 2.1, however, the interpretation of means of monthly values is limited for there is no indication of seasonal or diurnal fluctuation, or possible interaction of the different parameters (Hellawell, 1978).

Water flow rates fluctuated throughout the year. Annual mean daily flow rates for 1985 and 1986 were 7.501 and 8.152 cubic metres per second (cumecs) respectively. Flood levels rose to around 20 times the average flow rate whilst the low flow rates were approximately one-sixth of the average. The maximum daily flow was recorded in September (142.338 cumecs) and the minimum in June (1.666 cumecs).

Eels maintain station during periods of high flow rates by burrowing into the substrate and have a high tolerance of suspended solids although their olfactory sense of food location may be affected. High flow rates may dilute pollutants and increase the dissolved oxygen supply and some benthic organisms may be swept away. The efficiency of electrofishing may be affected by high flow rates and increased turbidity.

Water temperature varied within an annual range of 1 to 18.5 °C, where temperatures were highest in June (18.5 °C) and lowest in February (1 °C). It was calculated from continuous recording data that water temperature was more than 10 °C, when eel activity and feeding increases (Tesch, 1973), for less than 150 days between June 1985 and June 1986.

Water quality parameters reflected both seasonal and human influences. Pollution had a considerable effect on the river system where sewage, ferruginous, industrial and agricultural effluents were discharged. The reaction of the river was generally alkaline, within the range pH 6.9 - 7.9, which is well above the level that leads to acidification, that is, < pH 4.5, and within the normal range considered suitable for fish life, that is, pH 5 - 9 (ORSANCO, 1955 in Alabaster and Lloyd, 1980). Total ammonia reflects inputs of sewage effluent from activated sludge works, where bacteria oxidise the sewage, but do not break down ammonia. Maximum levels of total ammonia were high, although below the level toxic to fish, that is, 5 mg. l^{-1} but well above the level of a 'good fishery' of 1 mg. l^{-1}, as defined by the European Inland Fisheries Advisory Commission (EIFAC), (1970). Rising levels of both temperature and pH increase the toxic, un-ionized fraction of ammonia.

Suspended solid levels were extremely variable and within the range of 4.0 to 186.0 mg. l^{-1}, the higher values may reflect a shock pollution event such as spills of cattle and coal slurry or silage liquor. Foaming was a regular feature on the river in 1986 (Figure 1.2) which was caused by discharge from a detergent plant. Biological oxygen demand (BOD) values were within the range of 'fairly clean' to 'doubtful', as specified by the Royal Commission on Sewage Disposal (1912), as shown below:

Stream condition	**5 day BOD mg. l^{-1}**
very clean	1
clean	2
fairly clean	3
doubtful	5
bad	10

The respiratory requirements of abundant plant growth, flourishing due to high nutrient inputs from sewage works and agricultural run-off, may have reduced the dissolved oxygen during the night and produced supersaturation during the day, which might explain the maximum oxygen saturation of 137.7 %. There was dense algal growth during spring and summer, and sewage fungus growing at Site 1 during summer, when flow rates were low. Dissolved oxygen levels were generally high and

minimum levels were above the minimum constant value of 5 mg. l^{-1}, which satisfies most requirements providing other environmental factors are favourable, (Alabaster and Lloyd, 1980). Alkalinity and chloride values indicated that the buffering capacity of the water was high. However, the demands made on the river system were heavy due to the various effluent discharges and occasional shock pollution events and therefore the receiving water would have an important diluent effect.

TABLE 2.1 Seasonal variation in the abiotic conditions of the River Almond

	At time of sampling					Mean flow per month
Month	**Water Temp°C**	**Weather**	**Algal Growth**	**Flood Status**	**Pollution Status**	**Flowrate (cumecs)**
June (29.5.85)	17.0	Sunny/ dry	Heavy	Low	Sewage fungus Site1	. 2.449
July (9.7.85)	16.5	Sunny/ dry	Heavy	Low	Sewage fungus Site1	1.588
August (8.8.85)	13.0	Sunny/ dry	Medium	High	-	10.646
September (11.9.85)	12.5	Overcast/ dry	Medium	High	-	10.046
October (10.10.85)	10.0	Overcast/ dry	Low	High	-	21.051
November (5.11.85)	5.5	Overcast/ dry	Low	Medium	-	3.059
December (15.12.85)	1.5	Overcast/ dry	Low	Medium	-	10.277
January (26.1.86)	1.5	Overcast/ dry	Low	Medium	-	15.767
February (27.2.86)	1.0	Snow	Low	High	-	5.271
March (2.4.86)	5.0	Sunny/ dry	Low	High	-	9.460
April (28.4.86)	8.0	Rain	Low	Medium	-	10.905
May (2.6.86)	15.0	Sunny/ dry	Medium	Medium	-	8.278
June (3.7.86)	18.5	Sunny/ dry	Heavy	Low	Sewage fungus Site1 Foaming	3.838

2.3 FOOD AVAILABILITY

2.3.1 METHODS: Kick-sampling was used, as recommended by Macan (1958). The procedure involved kicking the river bed in a standard way for one minute whilst holding a net against the downstream side of the area to be sampled. The optimal mesh size is a compromise between the need to have a mesh fine enough to retain the organisms but not cause resistance to water flow. A fine mesh of 0.05 mm was required to retain naid worms. The net was held close to the river bed in an upstream direction to prevent loss of displaced animals in the outflowing current (Hynes, 1976). Because of the habitat variation each site was sub-divided into substrate types, that were predominantly rocky or muddy, and two samples from each were taken. Any stones, vegetation or detritus retained by the net were washed by hand to remove attached organisms. After collection the kick samples were transferred in jars to the laboratory where they were examined in an unpreserved state. The material was washed and put through a series of sieves of aperture sizes, 3.35 mm, 1.70 mm, 0.71 mm and 0.09 mm. The material retained by each sieve was transferred to a white enamel tray and sorted into different taxonomic groups, where the organisms in each group were identified to genus, or species where practicable. Specimens of Naididae often occurred as chains of individuals, the result of asexual budding, which were counted as one unit.

2.3.2 RESULTS: Species diversity was high and there were at least 50 different species of invertebrates present in the benthos, some present in all stages of their life cycle, such as the chironomids. The different stages have been considered separately and the fauna is also described as types of benthic organisms. The absolute and relative frequencies of each benthic organism were calculated from pooled monthly samples for each site. The relative frequency was computed by expressing the number of each organism as a percentage of the total fauna. Frequencies of occurrence of each benthic organism were sub-divided into 'common' and 'less common' types, where frequency exceeded 1 % in any sample and those where frequencies were less than 1 % in all samples. The seasonal variation in the composition of the bottom fauna is shown in Tables 2.2 and 2.3 for the 'common' and Tables 2.4 and 2.5 for the 'less common' types at both sites.

TABLE 2.2 Seasonal variation in the composition of 'common' benthic organisms at Site 1 (n=number, %=relative frequency, L=larvae, P=pupae, A=adult)

	J		J		A		S		O		N		D		J		F		M		A		M		J	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Chaetogaster sp.	-	-	-	-	-	-	-	-	-	-	2	0.4	7	1.1	1	0.1	1	0.04	1	0.03	-	-	41	2.6	5	0.14
Nais spp.	24	3.1	-	-	71	19.5	117	16.1	61	40.1	143	30.2	311	50.5	523	61.7	1586	62.1	2816	84.0	3914	89.1	816	52.6	161	4.6
Stylaria lacustris	77	10.0	1	0.05	176	48.4	171	23.5	19	12.5	42	8.9	15	2.4	3	0.3	-	-	-	-	-	-	6	0.4	23	0.7
Tubifex spp.	-	-	-	-	-	-	-	-	14	9.2	12	2.5	1	0.2	11	1.3	6	0.2	65	2.0	120	2.7	61	3.9	40	1.1
Helobdella stagnalis	4	0.5	-	-	-	-	8	1.1	-	-	2	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asellus aquaticus	134	17.4	13	0.7	13	3.6	6	0.8	2	1.3	30	6.3	2	0.4	5	0.6	6	0.2	47	1.4	11	0.3	19	1.2	2	0.06
Baetidae	4	0.5	24	1.2	14	3.9	95	13.1	9	5.9	34	7.2	87	14.1	117	13.8	37	1.5	42	1.3	66	1.5	8	0.5	27	0.7
Ecdyonuridae	-	-	2	0.1	-	-	-	-	-	-	-	-	5	0.8	5	0.6	2	0.08	1	0.03	3	0.09	-	-	1	0.03
Platambus maculatus(A)	6	0.8	-	-	5	1.4	1	0.1	-	-	-	-	1	0.2	-	-	-	-	-	-	1	0.01	1	0.06	2	0.06
Simulium sp.(L)	15	1.9	-	-	1	0.3	4	0.6	2	1.3	53	11.2	70	11.4	96	11.3	97	3.8	1	0.03	-	-	-	-	-	-
Chironomid spp.(L)	443	57.4	1868	93.9	31	8.5	232	31.9	6	4.0	69	14.6	108	17.5	77	9.1	722	28.3	294	8.8	199	4.5	536	34.5	2905	82.1
Chironomid spp.(P)	48	6.2	23	1.2	2	0.6	19	2.6	3	2.0	42	8.9	1	0.2	4	0.5	71	2.8	41	1.2	37	0.8	14	0.9	306	8.7
Chironomid spp.(A)	2	0.3	-	-	-	-	-	-	-	-	6	1.3	1	0.2	-	-	-	-	3	0.09	8	0.2	-	-	8	0.2
Chironomus sp.	-	-	6	0.3	-	-	-	-	-	-	6	1.3	-	-	2	0.2	2	0.08	5	0.15	5	0.1	2	0.12	16	0.5
Forcipomyia sp.	-	-	-	-	-	-	4	0.6	-	-	-	-	-	-	1	0.1	2	0.08	8	2.4	4	0.9	2	0.12	1	0.03
Terrestrial Insecta	1	0.1	1	0.05	3	0.8	9	1.2	6	4.0	2	0.4	-	-	-	-	-	-	1	0.03	-	-	3	0.2	6	0.17
Hydracarina	5	0.7	3	0.2	29	8.0	38	5.2	19	12.5	21	4.4	2	0.4	1	0.1	11	0.43	17	0.5	19	0.4	33	2.1	4	0.12
Limnaea pereger	-	-	9	0.5	4	1.1	6	0.8	-	-	-	-	1	0.2	-	-	-	-	-	-	1	0.1	1	0.06	13	0.4
Ancylastrum fluviatile	1	0.1	2	0.1	9	2.5	9	1.2	4	2.6	2	0.4	3	0.5	-	-	-	-	-	-	-	-	1	0.06	10	0.3

TABLE 2.3 Seasonal variation in the composition of 'common' benthic organisms at Site 2 (n=number, %=relative frequency, L=larvae, P=pupae, A=adult)

	J		J		A		S		O		N		D		J		F		M		A		M		J	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Chaetogaster sp.	-	-	-	-	-	-	-	-	-	-	34	2.7	8	0.3	17	0.9	5	0.2	3	0.05	-	-	21	0.9	90	4.3
Nais spp.	34	2.2	35	7.3	11	3.7	23	2.2	804	80.1	767	59.7	2504	81.5	1531	82.1	2518	80.7	6156	91.2	2594	84.2	1204	51.4	512	24.5
Stylaria lacustris	8	0.5	37	7.7	173	58.3	906	85.7	25	2.5	209	16.3	19	0.6	5	0.3	-	-	-	-	-	-	-	-	206	9.9
Tubifex spp.	-	-	8	1.7	10	3.4	-	-	70	7.0	80	6.2	376	12.2	16	0.9	85	2.6	387	5.7	231	7.5	509	21.7	126	6.0
Helobdella stagnalis	-	-	-	-	-	-	-	-	-	-	2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asellus aquaticus	50	3.2	22	4.6	48	16.2	2	0.2	4	0.4	21	1.6	12	0.4	-	-	3	0.09	6	0.09	13	0.4	11	0.5	6	0.3
Baetidae	1	0.06	2	0.4	5	1.7	52	4.9	16	1.6	22	1.7	72	2.3	32	1.7	31	1.0	41	0.61	20	0.7	23	0.9	34	1.6
Ecdyonuridae	-	-	5	1.0	-	-	-	-	1	0.1	4	0.3	5	0.16	5	0.3	1	0.03	1	0.02	1	0.03	-	-	7	0.3
Platambus maculatus(A)	3	0.2	7	1.5	6	2.0	-	-	-	-	-	-	1	0.03	-	-	1	0.03	-	-	2	0.06	-	-	1	0.05
Simulium sp.(L)	155	10.0	-	-	1	0.3	1	0.1	2	0.2	3	0.2	18	0.6	25	1.3	39	1.3	3	0.05	1	0.03	3	0.1	1	0.05
Chironomid spp.(L)	1079	69.6	292	60.7	28	9.4	55	5.2	47	4.7	71	5.5	-	-	217	11.6	400	12.8	78	1.2	81	2.6	527	22.5	941	45.0
Chironomid spp.(P)	197	12.7	3	0.6	10	3.4	11	1.0	13	1.3	31	2.4	28	0.9	-	-	17	0.6	11	0.2	34	1.1	15	0.6	144	6.9
Chironomid spp.(A)	7	0.5	-	-	-	-	-	-	-	-	7	0.6	4	0.1	-	-	-	-	-	-	43	1.4	3	0.1	11	0.5
Chironomus sp.	-	-	29	6.0	-	-	-	-	2	0.2	5	0.4	5	0.2	1	0.05	6	0.18	2	0.03	2	0.06	3	0.1	-	-
Forcipomyia sp.	3	0.2	-	-	-	-	-	-	1	0.1	4	0.3	-	-	4	0.2	7	0.2	16	0.2	12	0.4	6	0.3	-	-
Terrestrial Insecta	-	-	4	0.8	1	0.3	1	0.1	4	0.4	5	0.4	3	0.1	-	-	-	-	4	0.06	-	-	2	0.08	-	-
Hydracarina	8	0.5	9	1.9	1	0.3	2	0.2	3	0.3	2	0.2	2	0.06	5	0.3	4	0.13	31	0.6	36	1.2	7	0.3	3	0.1
Limnaea pereger	-	-	10	2.1	4	1.4	2	0.2	1	0.1	1	0.1	-	-	1	0.05	-	-	-	-	-	-	-	-	3	0.1
Ancylastrum fluviatile	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-	-	-	1	0.04	-	-

TABLE 2.4 Seasonal variation in the composition of 'less common' benthic organisms at Site 1

(n=number, %=relative frequency, L=larvae, P=pupae, A=adult)

	J		J		A		S		O		N		D		J		F		M		A		M		J	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Polyp	-	-	-	-	-	-	1	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydra vulgaris	-	-	-	-	-	-	1	0.14	2	1.4	2	0.4	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03
Dendrocoelom lacteum	-	-	-	-	-	-	2	0.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.06	-	-
Dugesia lugubris	-	-	-	-	-	-	2	0.28	1	0.7	-	-	-	-	-	-	1	0.04	-	-	-	-	-	-	-	-
Polycelis nigra	1	0.1	3	0.2	-	-	-	-	-	-	3	0.6	-	-	-	-	1	0.04	-	-	-	-	-	-	-	-
Lumbriculus variegatus	-	-	-	-	-	-	1	0.14	-	-	-	-	-	-	1	0.1	-	-	2	0.06	1	0.02	-	-	-	-
Glossiphonia complanata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.04	1	0.06	-	-
Herpobdella octoculata	1	0.1	-	-	-	-	1	0.14	-	-	-	-	-	-	1	0.1	-	-	3	0.09	2	0.04	1	0.06	-	-
Ostracod sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.06	-	-
Cyclops sp.	2	0.3	-	-	-	-	-	-	-	-	2	0.4	-	-	-	-	-	-	-	-	-	-	1	0.06	-	-
Gammarus pulex	1	0.1	1	0.06	-	-	-	-	-	-	-	-	-	-	-	-	2	0.08	-	-	-	-	1	0.06	-	-
Podura aquatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03
Stonefly	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.01	1	0.06	-	-
Hydroporus sp. (L)	2	0.3	-	-	-	-	-	-	1	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0.09
Hydroporus sp. (A)	1	0.1	-	-	1	0.3	-	-	1	0.7	-	-	1	0.2	-	-	-	-	-	-	-	-	-	-	-	-
Hydroptila sp.	-	-	3	0.2	1	0.3	1	0.14	1	0.7	-	-	-	-	-	-	1	0.04	1	0.03	-	-	1	0.06	-	-
Hydropsyche sp.	1	0.1	1	0.06	-	-	-	-	-	-	1	0.2	-	-	-	-	7	0.28	2	0.06	1	0.1	2	0.12	2	0.06
Dicranota sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-
Tipula sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-
Culex sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2	-	-	-	-	-	-	-	-	-	-	2	0.06
Chaoborus sp.	-	-	-	-	-	-	-	-	1	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limnophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.16	-	-	-	-	-	-	-	-
Terrestrial Insecta	-	-	-	-	1	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrobia jenkinsi	1	0.1	1	0.06	1	0.3	-	-	-	-	-	-	-	-	-	-	1	0.04	-	-	-	-	-	-	-	-
Aplecta fontinalis	-	-	-	-	2	0.6	-	-	-	-	1	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anguilla anguilla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.06	-	-

TABLE 2.5 Seasonal variation in the composition of 'less common' benthic organisms at Site 2 (n=number, %=relative frequency, L=larvae, P=pupae, A=adult)

	J		J		A		S		O		N		D		J		F		M		A		M		J	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Hydra vulgaris	-	-	-	-	-	-	2	0.2	1	0.1	2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	1	0.05
Dendrocoelom lacteum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.05	-	-	-	-	-	-	-	-	-	-
Dugesia lugubris	-	-	1	0.2	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-
Phagacata vitta	-	-	-	-	-	-	-	-	-	-	-	-	1	0.05	-	-	-	-	-	-	-	-	-	-	-	-
Polycelis nigra	-	-	1	0.2	-	-	-	-	-	-	1	0.1	-	-	1	0.05	-	-	-	-	1	0.03	1	0.04	-	-
Rhabdocoele	-	-	-	-	-	-	-	-	-	-	4	0.4	-	-	-	-	-	-	5	0.07	-	-	-	-	-	-
Nematode	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aeolosoma sp.	-	-	-	-	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eisenielia tetrahedra	-	-	-	-	-	-	-	-	2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lumbriculus variegatus	-	-	-	-	-	-	-	-	1	0.1	2	0.2	1	0.03	-	-	-	-	-	-	5	0.2	-	-	-	-
Slavina appendiculata	-	-	-	-	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glossiphonia complanata	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	2	0.1	1	0.03	-	-	-	-	1	0.04	-	-
Hemiclepsis marginata	-	-	4	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Herpobdella octoculata	-	-	1	0.2	-	-	-	-	2	0.2	-	-	3	0.1	2	0.1	-	-	2	0.04	2	0.06	1	0.04	-	-
Cyclops sp.	1	0.06	1	0.2	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	1	0.02	-	-	-	-	-	-
Gammarus pulex	-	-	3	0.6	1	0.3	-	-	1	0.1	-	-	-	-	1	0.05	-	-	-	-	-	-	1	0.04	-	-
Podura aquatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.04	-	-
Stonefly	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.05	3	0.09	1	0.02	-	-	-	-	-	-
Sialis sp.	-	-	1	0.2	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-	1	0.03	-	-	-	-
Hydroporus sp. (L)	1	0.06	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.2
Hydroporus sp. (A)	-	-	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Halmis sp.	-	-	-	-	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroptila sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.1	-	-	-	-
Dicranota sp.	-	-	-	-	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	1	0.04	-	-
Culex sp.	3	0.2	4	0.9	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-	-	-
Simulium sp. (P)	-	-	-	-	-	-	-	-	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chironomus sp. (P)	-	-	-	-	-	-	-	-	-	-	-	-	3	0.1	-	-	-	-	-	-	-	-	-	-	-	-
Limnophora sp.	-	-	-	-	-	-	-	-	-	-	2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrobia jenkinsi	-	-	2	0.4	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-	-	-	1	0.04	1	0.05
Aplecta fontinalis	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-	-	-	-	-	-	-	-	-
Pisidium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.03	-	-	-	-
Sphaerium sp.	-	-	-	-	-	-	-	-	1	0.1	-	-	1	0.03	-	-	-	-	-	-	-	-	-	-	-	-
Fish fry	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.2

FIGURE 2.1 Frequency of occurrence of major taxonomic groups at Site 1 and Site 2

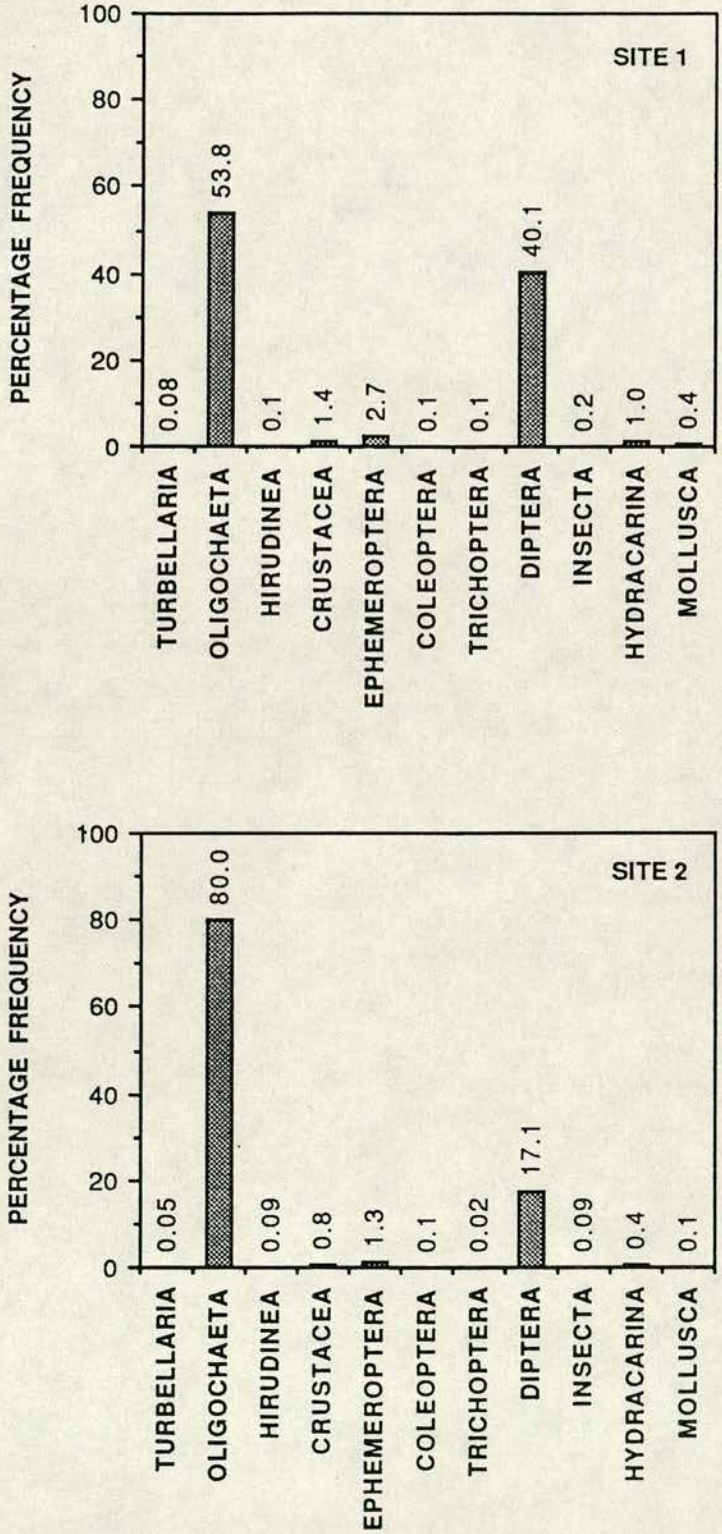


FIGURE 2.2 Frequency of occurrence of major taxonomic groups in each habitat type at Site 1 and Site 2

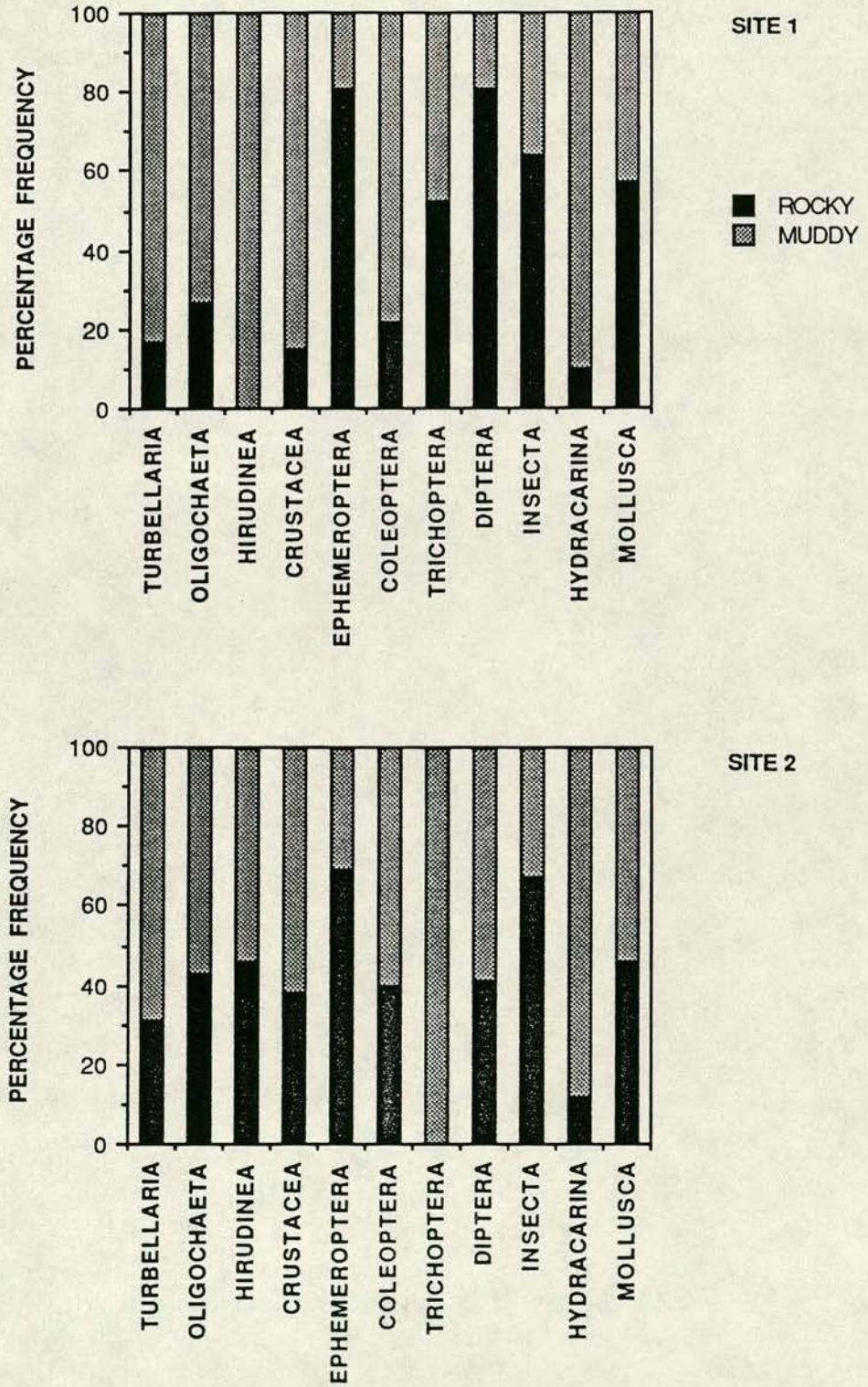
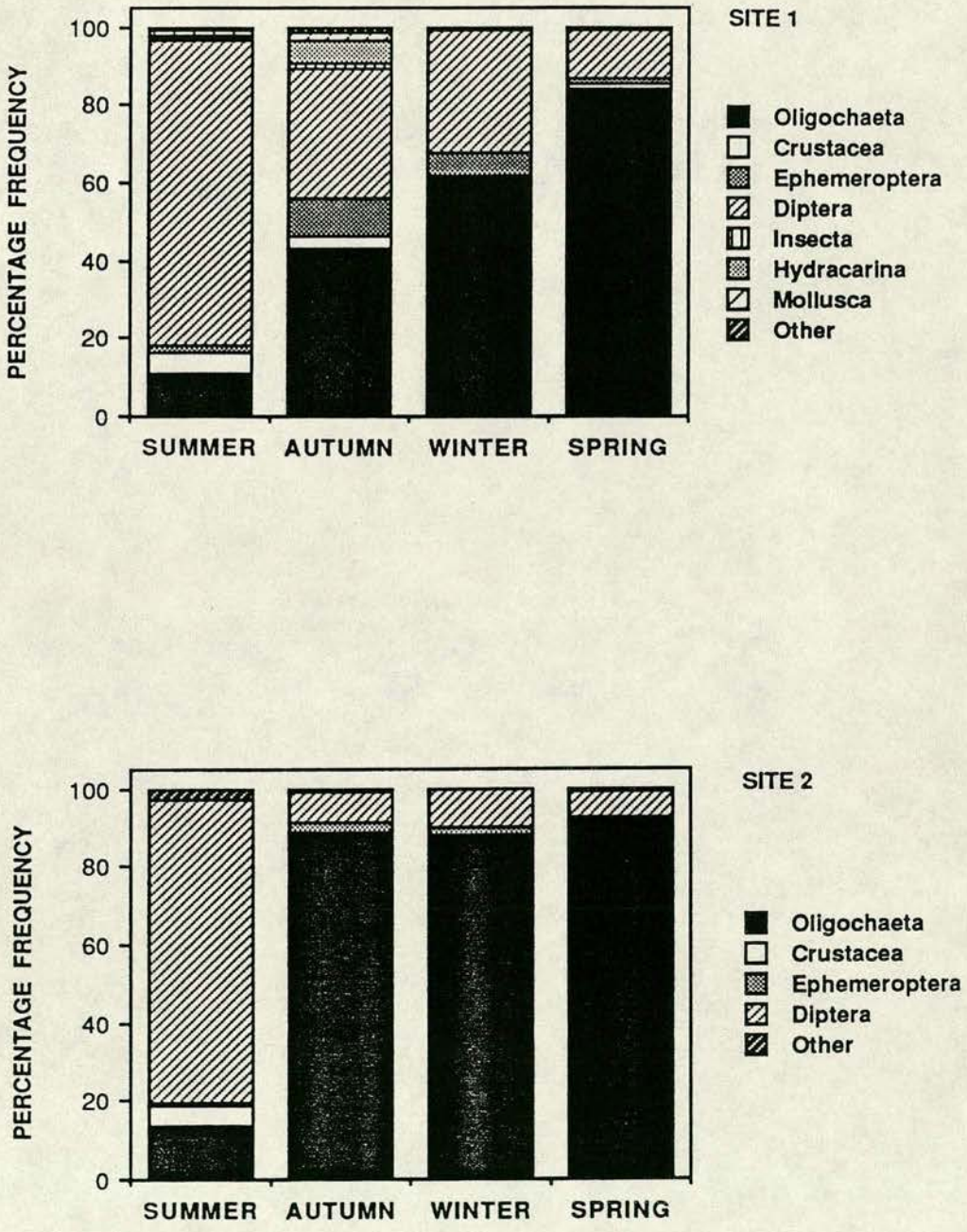


FIGURE 2.3 Seasonal variation in relative frequency of major taxonomic groups at Site 1 and Site 2



Site did not affect the occurrence of the 19 'common' benthic organisms which were present at both sites, whereas the 'less common' were more frequent at Site 2, that is 24 and 32 species at Sites1 and 2 respectively. At both sites the fauna were dominated by Oligochaeta and Diptera, with Ephemeroptera and then Crustacea as the next most common groups. Frequencies of Oligochaeta were particularly high at Site 2, where they made up 80% of the fauna. The frequencies of occurrence of the major taxonomic groups at both sites are shown in Figure 2.1.

Habitat did not affect the presence of the 19 'common' benthic organisms which were present in both the rocky and muddy habitats, but the 'less-common' were more numerous in the muddy, that is, 49 and 38 species in the muddy and rocky habitats respectively. The frequencies of occurrence of the major taxonomic groups in each habitat, where the frequency is expressed as a percentage of the total from both habitats over 13 months is shown in Figure 2.2. Oligochaeta and Crustacea were more abundant in the muddy habitat, whereas Diptera and Ephemeroptera were found more commonly in the rocky habitat.

Seasonal variation in the relative frequency of the major taxonomic groups at Site 1 and Site 2 is shown in Figure 2.3. The months were assigned to season as follows :- summer (June, July and August); autumn (September, October and November); winter (December, January and February) and spring (March, April and May). Seasonal changes in the absolute numbers of the four most common taxa and the total number of organisms in the other taxonomic groups are shown in Table 2.6.

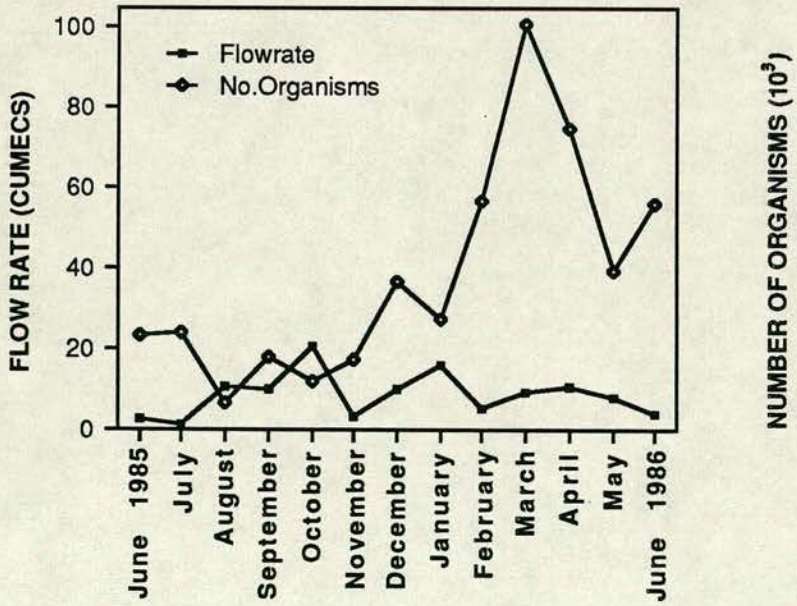
TABLE 2.6 Seasonal variation in absolute numbers of organisms

Organism	Summer	Autumn	Winter	Spring
SITE1				
Oligochaeta	349	583	2466	7843
Diptera	2439	447	1259	1161
Crustacea	164	40	15	80
Ephemeroptera	44	138	253	120
Others	103	142	31	94
SITE2				
Oligochaeta	316	2925	7085	11110
Diptera	1811	255	775	831
Crustacea	126	28	17	31
Ephemeroptera	13	89	144	85
Others	67	31	34	99

There was a marked increase in the frequency of Diptera during the summer, where they made up more than 77% of the fauna, which was due both to their increasing numbers and to the decrease in the numbers of Oligochaeta. Numbers of Diptera decreased suddenly in the autumn, and the group become secondary in abundance to Oligochaeta during the rest of the year. Crustacea and Mollusca were more abundant in summer and Ephemeroptera in autumn and winter. The total number of organisms was highest in the winter and spring at both sites, where Oligochaeta made up more than 60% of the fauna. However, the total number of organisms in the remaining taxa was lowest in winter.

Flow rate fluctuations affected the benthic fauna. A sudden spate, that is, an increase in flow rate following heavy rain or snow melt, had the effect of reducing the total number of organisms present in the samples. This was evident in August, October and January as shown in Figure 2.4.

FIGURE 2.4 Water flow rate and abundance of benthic organisms



Benthic organisms

Coelenterata

There were two representatives of coelenterates, a single polyp found at Site 1 and *Hydra vulgaris* present at both sites.

Turbellaria

Triclad flatworms were found more commonly in the muddy habitat where their preference for standing water was partly satisfied. *Dendrocoelom lacteum*, *Dugesia lugubris* and *Polycelis nigra* were more common than *Phagocata vitta.*

Oligochaeta

The most abundant oligochaete worms were species of Naididae. High frequencies were found in both habitats, with greatest abundance in the muddy, where *Nais spp.* and *Dero spp.* were common. *Stylaria lacustris* was present at similar frequencies in both rocky and muddy habitats, where it was found amongst vegetation. Another worm *Chaetogaster sp.* was found commonly in association with the gastropod *Limnaea pereger.* One species *Slavina appendiculata* was rarely found in the kick samples, but appeared with regularity in the eel gut contents (Chapter 2.4). This species has been described as common, but difficult to find, as it is usually covered by a layer of detritus (Clegg, 1963). *Tubifex* worms were most commonly found in the muddy habitat but were subject to fluctuation in frequency. In polluted water they can be found in large colonies, which are susceptible to being swept away by floods. This was not apparent in the River Almond, where numbers of worms decreased during periods of low water flow rates. Representatives of other families of Oligochaeta were *Lumbriculus variegatus* and *Eiseniella tetrahedra*, which were found at low frequencies in the mud, and near the riverbank in particular.

Hirudinea

Leeches were more commonly found in the muddy habitat. *Helobdella stagnalis* and *Herpobdella octoculata* were the most numerous whilst *Glossiphonia complanata* and the fish leech *Hemiclepsis marginata* were rare.

Crustacea

The most common species of crustacean was *Asellus aquaticus* which was more common in the muddy habitat. It is tolerant of low oxygen concentrations and high organic matter content, whilst the less common *Gammarus pulex* is not. Rarer still were *Cyclops* and a species of Ostracoda.

Diptera

The increase in chironomid larvae during springtime can in part be attributed to long periods with uniform water levels (Grimas, 1963). Large numbers were found in association with green algae or macrophytes, which was also found by Hussein

(1983). One chironomid *Forcipomyia*, when present, was consistently found among the blanket weed *Cladophora glomerata*. Another distinctive chironomid *Chironomus thummi* (=*riparius*) was more commonly found in the muddy habitat, where it is adapted to lower oxygen levels due to its blood haemoglobin. The short pupal life of many Dipterans may explain the low pupal frequencies, for example, *Simulium* pupal life, lasts only 2-3 weeks. Numbers of *Simulium* larvae varied greatly, one explanation is due to their tendency to clump together in high densities, where stream conditions suit their method of feeding, that is, a specialised type of filter feeding on mainly single-celled plants (Morgan and Egglishaw, 1965). Other Dipterans present were:- *Dicranota* and *Tipula* larvae (Tipulidae); *Culex* larvae, pupae and adults and *Chaoborus* larvae (Culicidae) and *Limnophora* larvae (Anthomyiidae), which is another species commonly found amongst plant growth.

Ephemeroptera

Apart from the Diptera, ephemeropteran nymphs were the most common order of Insecta. They were identified to family level only, that is, Baetidae and Ecdyonuridae. The former were the most common and both were most abundant in the rocky habitat.

Other Orders of Insecta

Less commonly represented in the bottom fauna were: caddis fly nymphs, both cased *Hydroptila sp.* and caseless *Hydropsyche sp.* (Trichoptera); *Hydroporus sp.* larvae and adults, *Platambus maculatus* adults and *Helmis sp.* larvae (Coleoptera); *Podura aquatica* adults (Collembola); stone-fly nymphs (Plecoptera) and Alder-fly nymphs, *Sialis sp.* (Neuroptera). There was an allochthonous input of insects which are referred to as 'terrestrial Insecta' and not identified further.

Hydracarina

Species of water-mites were not identified beyond suborder level. They were present throughout the year and were most common in muddy habitat. Their numbers fluctuated and in one sample contributed to 12.4 % of the benthic fauna.

Mollusca

There were six species of mollusc present but none was particularly common. *Limnaea pereger* was most common and was equally common in either habitat. *Ancylastrum fluviatile* has a low tolerance for suspended matter and was more common in the rocky habitat. The other species were *Hydrobia jenkinsi*, *Aplecta fontinalis*, *Pisidium sp.* and *Sphaerium sp.*

Fish

Fish fry were present in the kick samples on one occasion and one glass eel, *Anguilla anguilla* on another. Further details of the fish species present, collected

whilst electrofishing, are given in Chapter 4.2.5.

2.3.3 DISCUSSION: The benthic fauna was dominated by pollution tolerant organisms such as chironomids, oligochaete worms and the isopod *Asellus aquaticus,* whereas pollution sensitive organisms such as species of Trichoptera and Plecoptera were rare. Pollution indices, such as the Trent Biotic Index (Woodiwiss, 1964) which gives the River Almond a 'clean' rating of IX, do not take into account the relative frequencies of pollution tolerant and sensitive species.

Site and habitat variation affected distribution and occurrence of organisms, with greatest species diversity at Site 2, although frequencies were low except for the ubiquitous oligochaetes and chironomids. Seasonal changes affected the amount of detrital input, which was increased with die-back of macrophytes and leaf-fall from trees, in the autumn. This coincided with increasing numbers of organisms in winter and spring (Varley, 1967), although species diversity was greatest in the summer, unlike Varley (1967), but similarly found by Frost (1945). There is a seasonality in the life-cycles of most organisms which affects abundance. Crustacea breed in summer, and molluscs in the spring and summer, which probably explains the increasing abundance in the River Almond. Many insects are present as eggs or aerial adults in summer and frequencies of nymphs and larvae increase in the autumn, winter and spring, which may explain the greater numbers of Ephemeroptera in the autumn and winter. Organisms having short life-cycles, such as chironomids, increase their numbers rapidly in spring and early summer due to the rising water temperatures, whilst oligochaete numbers increase in winter, probably because of the additional detrital input.

2.4 FOOD ORGANISMS

2.4.1 METHODS: Quantitative samples of eels, elvers (pigmented year 0+) and glass eels (unpigmented year 0+) were taken at monthly intervals. Weight, minus the gut contents, and length measurements were recorded, before freeze storage until further examination. Because feeding activity of the eel is mainly nocturnal (Sinha and Jones, 1975) specimens were collected in the early morning to minimise the effects of digestion. The examination of intestinal contents can be a useful indication of the constitutents of a previous meal (Opuszynski and Leszczynski, 1967 in Tesch, 1973), or of food organisms generally, when there are no stomach contents. This was useful in the present study during periods of low feeding activity.

Index of fullness

During dissection of each fish the fullness of each stomach was recorded on a scale of 0-5 as an indication of feeding activity (Ball, 1961) as follows:

Visual estimate of fullness	Points
Empty	0
Trace	0.5
1/4 full	1
1/2 full	2
3/4 full	3
full	4
distended	5

Stomach fullness index

The stomach fullness index, expressed as the mean number of points scored by all the stomachs examined in each monthly sample, at each site, was calculated for eels, elvers and glass eels.

Gut content status

The monthly gut content status of the eels and elvers at both sites was determined by calculating the percentage with stomach contents, stomachs containing a trace of food (0.5 of Ball's index), empty stomachs with food remains in the intestine and completely empty guts.

Stomach and intestinal contents

The stomach (cardiac-pylorus delimiters) and intestine (pylorus-anus delimiters) were sectioned longitudinally and the contents of each extruded into

separate petri dishes. The contents were swirled in water before examination under a binocular microscope. The stomach contents were sorted into different categories and the food organisms counted and identified to species level where possible. Most organisms were easily identifiable, which indicated minimal digestion and the likelihood that the stomach contents represented one meal or feeding session. It has been estimated that digestion of chitinous organisms takes 10 hours (Sinha and Jones, 1975). The intestinal contents were similarly sorted, but not counted, as the organisms were in a more particulate form. A smear of material in suspension was taken from stomach and intestinal contents, for examination under the light microscope, for detection of chaetae and bristle hairs from oligochaete worms. The presence of filamentous algae, plant debris and stones in the stomach contents was recorded.

The amount and variety of food consumed was analysed in terms of frequency of occurrence and relative abundance and no attempt to measure volume was made. Hynes (1950) in his review of methods of assessment of food of fresh water fish concluded that all methods substantially give the same results, if each food item was shown as a percentage of the food eaten.

Frequency of occurrence of food organisms was calculated by determination of the number of stomachs containing a particular food organism, expressed as a percentage of all the stomachs that contained food. This represents the proportion of the population feeding on the particular organism and will include unquantifiable food items, such as oligochaetes, which may be damaged on egestion or undergo rapid digestion. Oligochaetes were recorded as nearly always being broken-up by Hynes (1950).

Relative abundance of food organisms was calculated by determination of the number of each food organism expressed as a percentage of all food items. It is an estimate of the relative abundance of the particular food item in the diet and allows comparison with relative abundance in the benthos, and thereby the degree of food selection, but will not give a true value of the relative abundance of Oligochaeta.

2.4.2 RESULTS: The composition of the diet at both sites is shown for eels in Table 2.7 and for glass eels and elvers in Table 2.8. Diet was more varied at Site 1 in all stages of eels examined, that is, 32 and 16 types of food organisms at Sites 1 and 2 respectively. This was in contrast to the lower species diversity in the benthos, although the frequencies in the benthos were higher at Site 1. Eels at Site 1 were significantly smaller than Site 2, that is, median = 22.8 cm and 25.8 cm respectively ($p<0.001$; Mann-Whitney U -test). The frequency of occurrence of the most common

taxonomic groups in the diet of eels is shown in Figure 2.5 and glass eels and elvers in Figure 2.6 for both sites. Diptera and Oligochaeta are predominant in the diets at all stages, followed by Crustacea and Ephemeroptera in the eels and glass eels, and Ephemeroptera and terrestrial Insecta in the elvers.

TABLE 2.7 Composition of the diet of eels at Site 1 and Site 2

(n f = number of stomachs(n) frequency of occurrence(f), n a = number of organisms(n) frequency of abundance(a), L=larva, P=pupa, A=adult)

Food organism	Site 1				Site 2			
	n	f	n	a	n	f	n	a
Dendrocoelom lacteum	2	0.5	4	0.1	1	1.0	1	0.1
Dugesia lugubris	2	0.5	3	0.1	-	-	-	-
Slavina appendiculata	62	14.1	6	0.2	10	10.2	-	-
Nais spp.	40	9.1	3	0.1	3	3.1	-	-
Stylaria lacustris	18	4.1	-	-	10	10.2	-	-
Tubifex spp.	2	0.5	-	-	-	-	-	-
Lumbriculus variegatus	3	0.7	3	0.1	1	1.0	1	0.1
Hemiclepsis marginata	1	0.2	1	0.03	-	-	-	-
Herpobdella octoculata	1	0.2	1	0.03	-	-	-	-
Asellus aquaticus	67	15.2	553	18.4	14	14.3	83	10.7
Gammarus pulex	1	0.2	1	0.03	-	-	-	-
Cyclops sp.	-	-	-	-	-	-	-	-
Plecoptera	1	0.2	1	0.03	-	-	-	-
Ephemeroptera	42	9.5	152	5.1	5	5.1	21	2.7
Hydroporus sp. (L)	2	0.5	5	0.2	1	1.0	1	0.1
Hydroporus sp. (A)	2	0.5	3	0.1	-	-	-	-
Lepidoptera (L)	1	0.2	1	0.03	-	-	-	-
Trichoptera	18	4.1	34	1.1	1	1.0	1	0.1
Culex sp.	2	0.5	2	0.07	-	-	-	-
Simulium sp. (L)	27	6.2	170	5.7	3	3.1	13	1.7
Simulium sp. (P)	1	0.1	1	0.03	-	-	-	-
Chironomid spp. (L)	78	17.7	1812	60.2	25	25.5	512	66.1
Chironomid spp. (P)	47	10.7	210	7.0	19	19.4	121	15.6
Chironomid spp. (A)	1	0.2	5	0.2	2	2.0	6	0.8
Forcipomyia sp.	2	0.5	2	0.07	1	1.0	1	0.1
Chironomus sp.	4	1.0	4	0.1	-	-	-	-
Terrestrial Insecta	4	1.0	4	0.1	-	-	-	-
Hydracarina	1	0.2	5	0.2	-	-	-	-
Limnaea pereger	6	1.4	19	0.6	2	2.0	13	1.7
Ancylastrum fluviatile	1	0.2	2	0.07	-	-	-	-
Anguilla anguilla	2	0.5	2	0.07	-	-	-	-

TABLE 2.8 Composition of the diet of elvers and glass eels at Site 1 and Site 2 (n f = number of stomachs(n) frequency of occurrence(f), n a = number of organisms(n) frequency of abundance(a), L=larva, P=pupa, A=adult)

Food organism	SITE1								SITE2							
	Elver				Glass eel				Elver				Glass eel			
	n	f	n	a	n	f	n	a	n	f	n	a	n	f	n	a
Slavina appendiculata	1	6.3	1	5.6	58	35.6	-	-	2	40	2	20	26	61.9	-	-
Nais spp.	2	12.5	2	11.1	27	16.6	-	-	1	20	1	40	1	2.4	-	-
Tubifex spp.	-	-	-	-	3	1.8	-	-	-	-	-	-	-	-	-	-
Lumbriculus variegatus	1	6.3	1	5.6	-	-	-	-	-	-	-	-	-	-	-	-
Cyclops sp.	-	-	-	-	3	1.8	3	0.7	-	-	-	-	-	-	-	-
Ephemeroptera	3	18.8	4	22.2	5	3.1	6	1.3	-	-	-	-	1	2.4	2	6.9
Hydroporus sp. (L)	-	-	-	-	2	1.2	2	0.5	-	-	-	-	-	-	-	-
Simulium sp. (L)	3	18.8	3	16.7	-	-	-	-	-	-	-	-	1	2.4	1	3.5
Chironomid spp. (L)	5	31.3	6	33.3	54	33.1	409	91.1	1	20	1	20	11	26.2	24	82.8
Chironomid spp. (P)	-	-	-	-	11	6.8	29	6.5	-	-	-	-	1	2.4	1	3.5
Chironomid spp. (A)	-	-	-	-	-	-	-	-	-	-	-	-	1	2.4	1	3.5
Terrestrial Insecta	1	6.3	1	5.6	-	-	-	-	1	20	1	20	-	-	-	-

FIGURE 2.5 **Frequency of occurrence of major taxonomic groups in the diet of eels at Site 1 and Site 2**

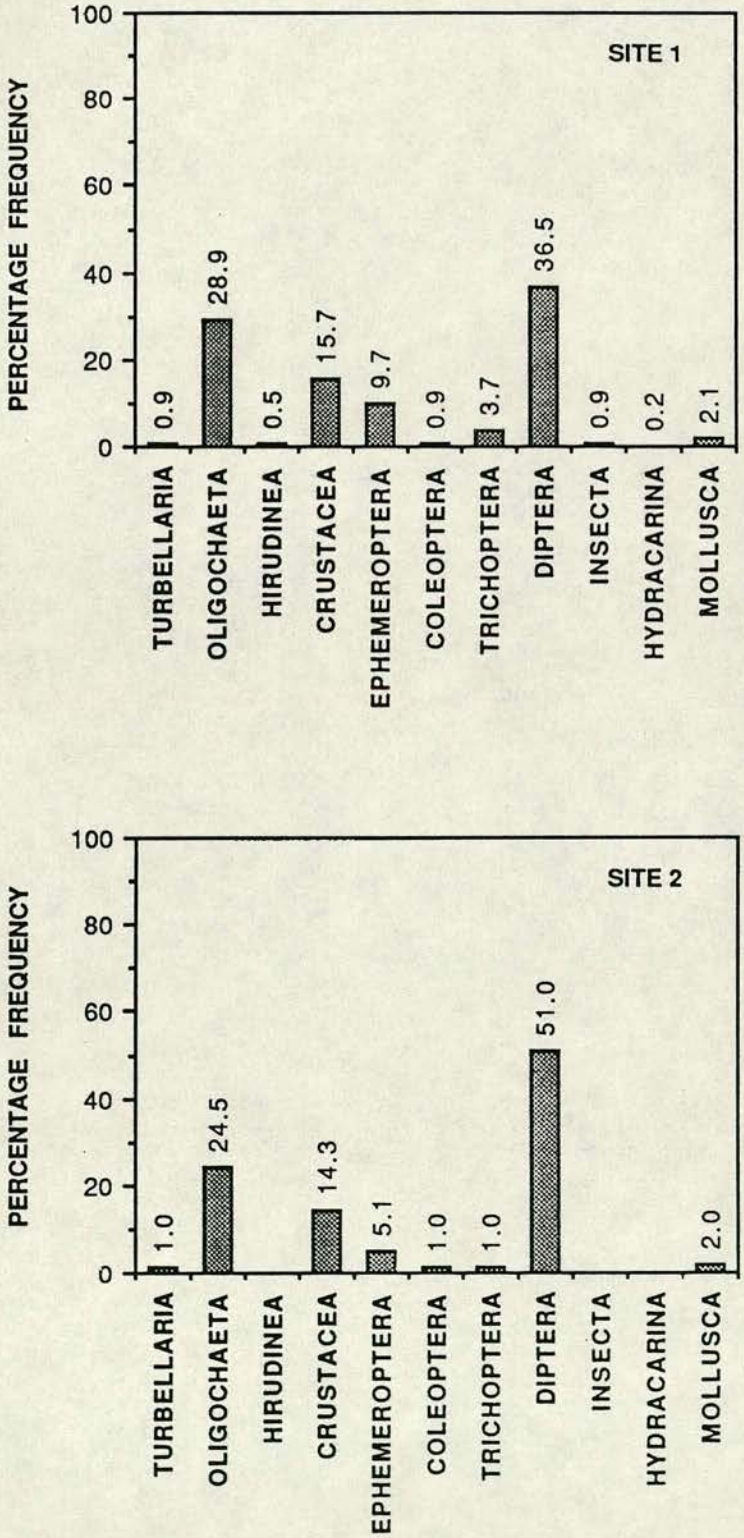
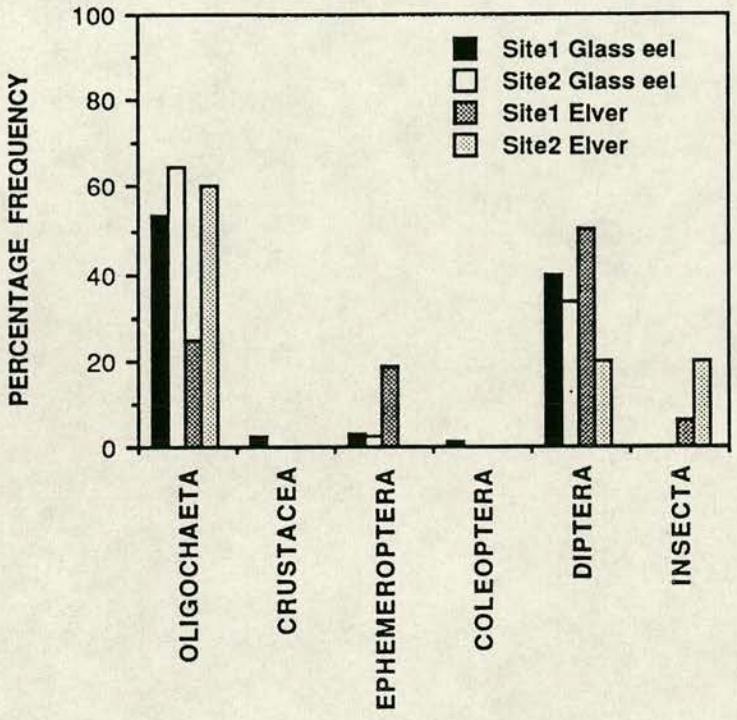


FIGURE 2.6 Frequency of occurrence of major taxonomic groups in the diet of glass eels and elvers at Site 1 and Site 2



Stomach fullness indices are shown in Figure 2.7 where the water temperature at the time of sampling is indicated. The water temperature was highest in June (18.5 °C) and lowest in February (1.0 °C). Feeding activity of eels was closely linked to water temperature and peaked in June, lessening gradually during late summer and autumn to virtually zero during the winter, before increasing again in early spring. There was little feeding below 10 °C. This pattern was seen at both sites although the fullness indices achieved were lower at Site 2. Feeding intensity of glass eels peaked in May.

Gut content status is shown in Figure 2.8. The number of empty stomachs was highest in the winter months, which extended from October to March at Site 2 and from January to February at Site 1, although there were eels with empty stomachs in spring and summer, at both sites. There were food remains in the intestines of individual eels in all monthly samples, and traces of food in the stomachs of a high percentage of winter samples. The frequency of occurrence of the food organisms in the intestine was similar to that found in the stomach contents (Appendix 2.2). There were remains of various foods in the intestine of eels with empty stomachs, in January at Site 1 and October at Site 2, as shown in Figure 2.9.

FIGURE 2.7 Index of stomach fullness of eels, and water temperature at time of sampling, at Site 1 and Site 2

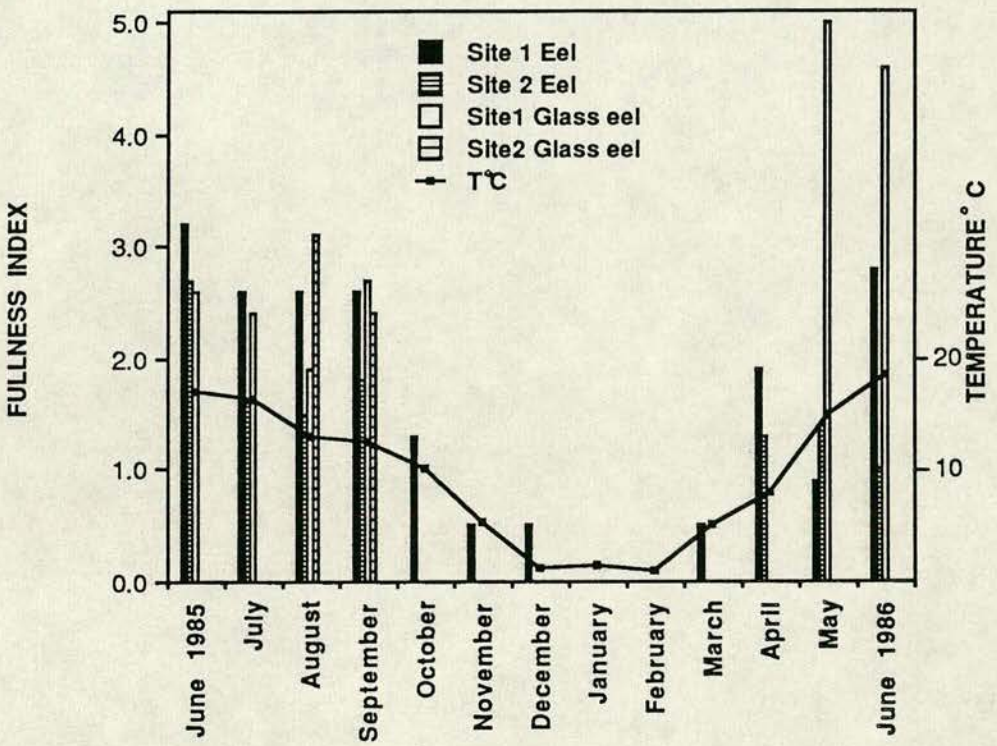
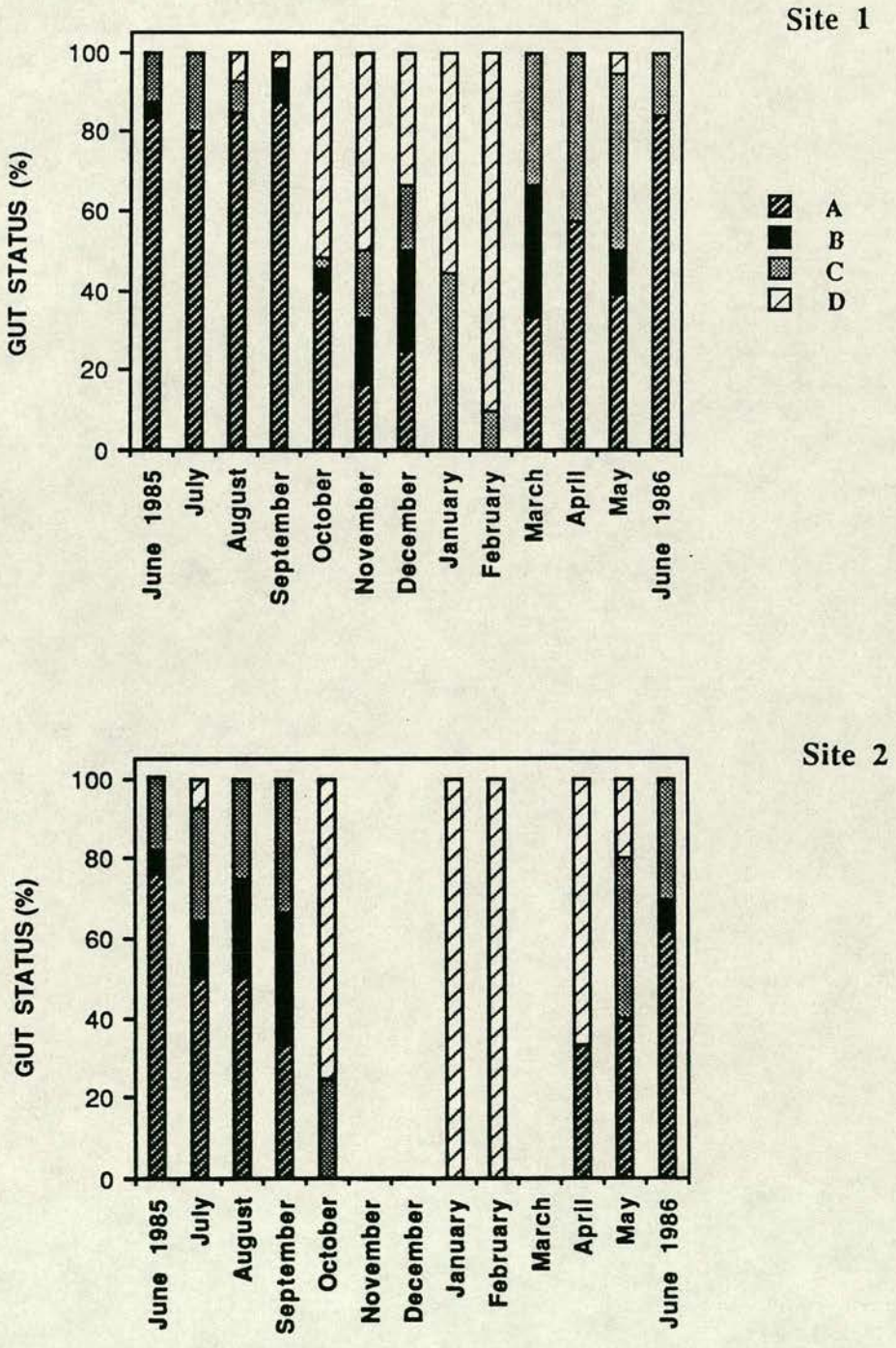


FIGURE 2.8 **Variation in the gut content status of eels and elvers at Site 1 and Site 2** (A=stomach contents, B=trace in stomach, C=empty stomach with intestinal contents, D=empty gut)



Seasonal variation in the frequency of occurrence of the main taxonomic groups of food organisms, in the stomach contents, throughout the sampling period is shown in Figure 2.9. Oligochaeta and Diptera were the most common food taxa throughout the year, the former predominating from August to May, with Diptera becoming most abundant in June and July. Crustacea were more abundant in the diet from April to August, whereas Ephemeroptera were more common in September at Site 1, but were rare throughout the year at Site 2. Similarly, Coleoptera, Trichoptera and Mollusca were rare at Site 2, but were present in higher frequencies during the summer at Site 1. Hirudinea and Hydracarina were found in the diet at Site 1 but were absent from Site 2, whereas Turbellaria were rare at both sites, and terrestrial Insecta were more commonly found at Site 1. The diet of elvers shows a similar change from Oligochaeta to Diptera in June, but thereafter reverts to higher frequencies of oligochaetes, as shown in Figure 2.10.

Cannibalism was recorded on two occassions, where elvers were found in eels of 22.6 cm and 26.0 cm, in June and August respectively.

Omnivory was more common. Of the 177 eel stomachs containing food, filamentous algae *Cladophora glomerata* and plant debris were present in 5 % and 16 % respectively and small stones were present in 11 %. All were found throughout the size range of eels and were most common in eels between 25.0 to 30.0 cm and least common in glass eels. In the glass eels there was no filamentous algae present although plant debris and stones were present in 4 % and 1 % respectively. Plant material and stones have previously been found in the stomach contents and especially in larger individuals (Rasmussen and Therkildsen, 1979).

FIGURE 2.9 Seasonal variation in the diet of eels at Site 1 and Site 2

(stomach contents unless marked otherwise)

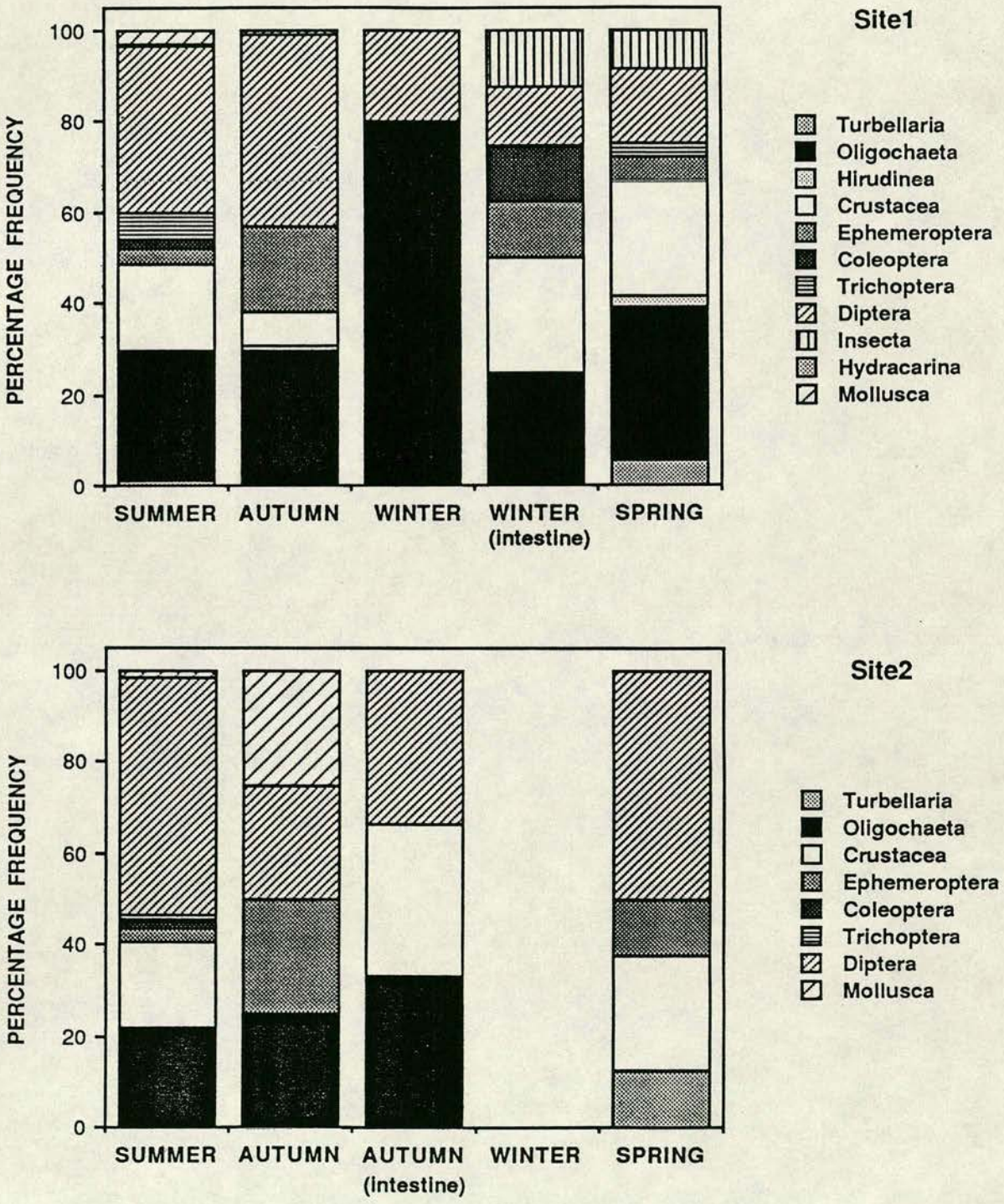
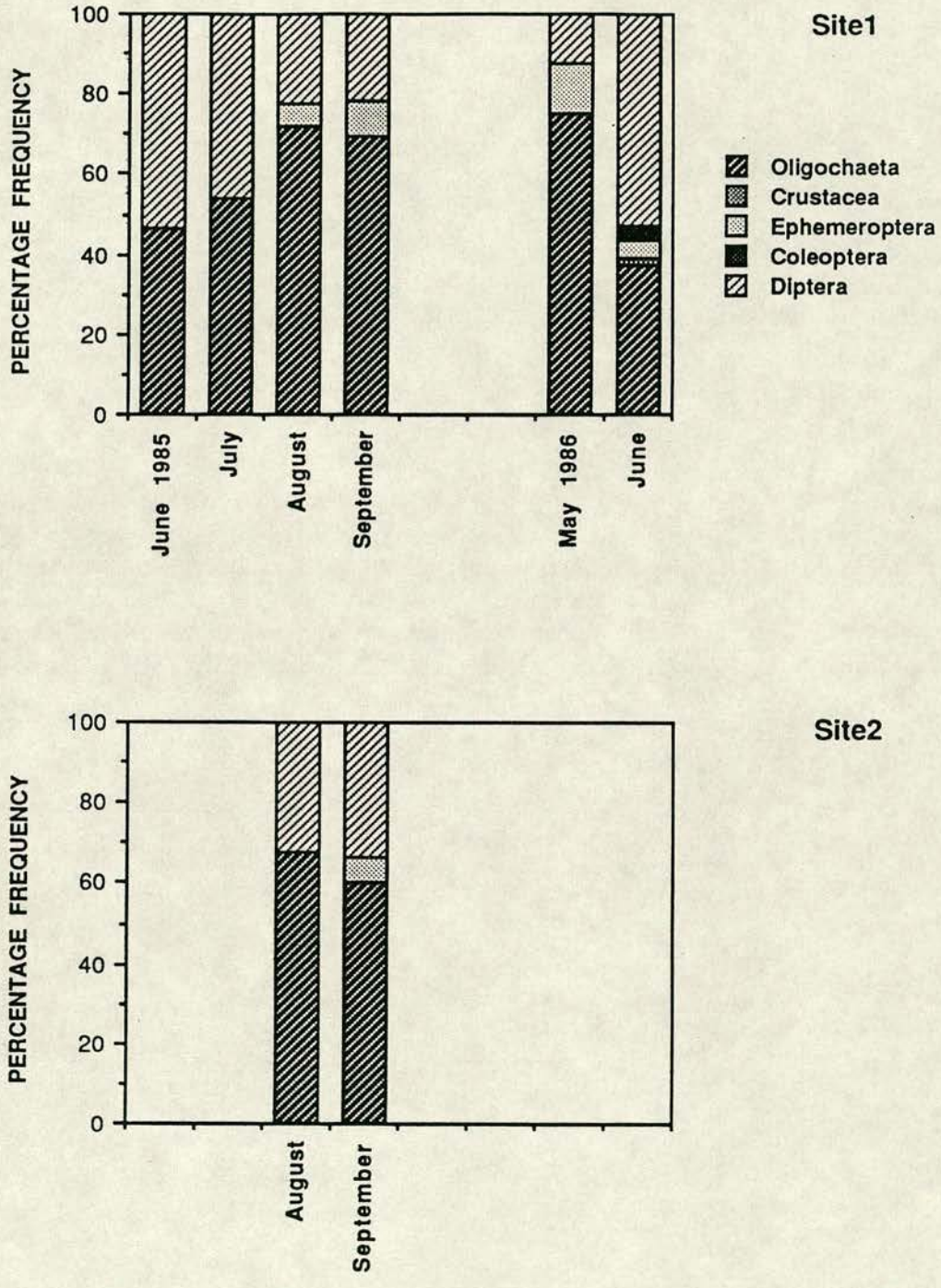


FIGURE 2.10 Seasonal variation in the diet of elvers at Site1 and Site2



2.4.3 DISCUSSION: The diet of eels was largely determined by the frequency of the food organisms in the benthos and consisted mainly of naid worms and chironomid larvae. With rising water temperature in the spring, the numbers of chironomid larvae in the benthos and the proportion in the diet increased during June and July, where otherwise oligochaetes dominated the benthos and diet from August to May. The number of other organisms in the benthos increased during the summer (Table 2.6) and were more abundant in the eel diet (Figure 2.9), especially Crustacea, whereas Ephemeroptera were more plentiful in the benthos and eel diet during the autumn.

The indices of stomach fullness (Figure 2.7) and gut content status (Figure 2.8) indicate that feeding activity is related to water temperature and that above 10 ° C there is increased activity. Some eels at Site 1 had food in their guts from November to March, whereas eels at Site 2 had completely empty guts during this time. The analysis of gut content status demonstrates that there is some feeding activity throughout the year, under certain conditions. The smaller size of the eels and their proximity to the sea at Site 1 may explain the difference in feeding activity between the sites.

The presence of eels with empty stomachs and intestines during summer suggests that some eels may not eat daily, and that they may fast for 2 to 3 days (Moriarty, 1983). Whereas some eels are described as being 'constant nibblers' (Sinha, 1965) which may be the situation in some of the eels at Site 1. There were undigested food organisms, for example, *Slavina appendiculata* and *Asellus aquaticus*, in the intestinal contents of eels in the River Almond, which suggests a more voracious type of feeding. Thus, it seems likely that there are eels following different lifestyles (Moriarty, 1987a) which will also be reflected in the various growth rates of individual eels.

2.5 EEL MORPHOLOGY

2.5.1 INTRODUCTION: Eels have very small heads suited to burrowing in mud and sand and creeping into small holes. Their mouths are correspondingly small, which limits the size of the food item, and often results in food being bitten or ripped into smaller pieces before swallowing. Apart from a direct proportionality between body size and head size eels may be 'narrow-headed' or 'broad-headed'. 'Broad-headed' eels have short, blunt, almost depressed snouts with a wider distance between the eyes and nostrils and more pronounced lower lip than 'narrow-headed' eels whose snout is elongated and narrow. These differences are distinct from those involved with secondary sexual characteristics (Nordquist, 1917 in Tesch, 1973). Opinion has developed from the suspicion that two distinct species were involved (Risso, 1826 in Tesch, 1973) to consideration of the 'broad-headed' and 'narrow-headed' eels as extremes of a single modal type (Frost, 1945; Matsui, 1952 and Thurow, 1957), which have been further distinguished as rapidly and slow growing respectively (Matsui, 1952). Further debate ensued as to when differentiation occurred, whether during the course of ontogeny (Törlitz, 1922) or as a result of environmental factors (Thurow, 1957).

Increase in mouth size corresponding with increasing head width allows larger food items to be taken. It has been reported that 'broad-headed' eels were more piscivorous (Micheler, 1967; Draganic, 1962) and ate more molluscs (Micheler, 1967 and Opuszynski and Leszczynski, 1967 in Tesch, 1973). In contrast, worms were more commonly found in the stomach contents of 'narrow-headed' eels. Furthermore, the differences in food items taken by 'broad-headed' and 'narrow-headed' eels were found to be similar to those between small and large eels. No differences, however, were found in the feeding habits of the two forms in other studies (Frost, 1946).

The differences in the feeding habits of the two forms led to speculation as to the effect of environmental factors on head shape and even a Lamarckian argument that head shape might be determined by prey size (Thurow, 1957). This was countered by Opuszynski, 1963, who reasoned that a certain head shape would predispose the eel to a particular diet, for example, 'broad-headed' eels would become predators more easily. This section investigates the head shape of eels in the River Almond and examines any affect of head shape or body size on diet composition.

2.5.2 METHODS:

Head shape affects mouth size, which is also proportional to the distance between the eyes, the interpupillary distance (Törlitz, 1922). A study of

morphometrics of cultured *Anguilla anguilla* showed that jaw gape was variable, but mouth width corresponded well with the upper limit of optimum size of food particles (Knights, 1982). Measurement of interpupillary distance was achieved with vernier calipers, to the nearest 0.01 cm, but was not practicable for eels under 1g in weight. The effect of proportional increase of mouth size with increasing length of eel was removed by expressing the interpupillary distance as a ratio of the total length or head length.

Total length was measured using a measuring board to the nearest 0.1 cm and head length, that is, from the tip of the snout to the insertion of the pectoral fin, was measured with vernier calipers to the nearest 0.01 cm. Elvers were allocated to two size classes; year 0+, which included glass eels and pigmented elvers, and year 1+, and eels were allocated to 5 cm size groups between 10 cm and 50 cm. The composition of the diet of each group was determined by examination of the frequency of occurrence of each food organism.

Size of the food organism was measured with a binocular microscope, fitted with an eyepiece graticule, to the nearest 0.01 cm. The length and width of a number of the 'common 'food organisms were measured, excluding appendages, from the stomach contents of eels in a range of size groups, that is, *Asellus aquaticus*, Baetidae, chironomid larvae and pupae, *Simulium sp.* pupae and *Limnaea pereger*. Also a few of the 'less common' food organisms were measured, that is, *Dendrocoelom lacteum*, *Herpobdella octoculata* and caddis fly nymphs.

2.5.3 RESULTS:

Effect of head shape

Head width plotted against total length and head length, followed a normal distribution. Thus, it was evident that the River Almond eel population was made up of one form of head shape. Coefficient of variation (CV) of head width was less than total length, that is, 33.5 % and 45.5 % respectively (CV=100 x std.dev. / mean).

Values of head width as a percentage of head length were:- mean 20.2 % (range 13.7 - 29.5 %) and as a percentage of total length were mean 2.4 % (range 1.7 - 3.0 %). This corresponds with values for 'narrow-headed' eels of 2.5 % and for 'broad-headed' eels of 4.7 % for total length (Walter, 1910 in Tesch, 1973; Törlitz, 1922). The population of River Almond eels fit into the 'narrow-headed' category and any differences in head shape and thus, mouth size, will be due to a direct proportionality with body size.

Effect of size of eel

The percentage composition of the major taxonomic groups in the diet of each size group of eels is shown in Figure 2.11. The diet of the elvers is restricted to ten types of food organisms and there is a slight difference in the organisms in pigmented elvers and glass eels (Table 2.8), that is, chironomid adults but not terrestrial Insecta are present in the diet of glass eels whilst the converse occurs in the pigmented elvers. *Cyclops* were only present in the diet of glass eels. There was a tendency towards a greater species diversity in the diet of eels in intermediate size groups, for example, eels from 25.1-35.0 cm had nearly twice the number of food organisms compared with other size groups. Diptera occurred in high frequencies in all size groups under 45 cm length and were absent in larger eels. Frequencies of oligochaetes declined with increasing size of eel, with a corresponding increase in other taxa, especially Crustacea, and molluscs were only present in eels over 15.0 cm.

Effect of size of food organism

The range in length and width of the food organisms measured are shown in Table 2.9.

TABLE 2.9 Size of food organism (L=larva, P=pupae, n=sample size)

Food organism	n	Size range (cm) Length	Width
Dendrocoelom lacteum	1	2.40	0.35
Caddis fly nymph	4	1.60 - 0.16	0.28 - 0.04
Baetidae	10	1.14 - 0.30	0.30 - 0.04
Asellus aquaticus	15	1.08 - 0.08	0.34 - 0.05
Chironomid (L)	21	1.00 - 0.46	0.10 - 0.04
Chironomid (P)	15	0.62 - 0.26	0.12 - 0.06
Herpobdella octoculata	1	0.57	0.35
Simulium spp. (P)	8	0.53 - 0.12	0.11 - 0.05
Limnaea pereger	9	0.46 - 0.34	0.32 - 0.18

FIGURE 2.11 Effect of size of eel on diet composition

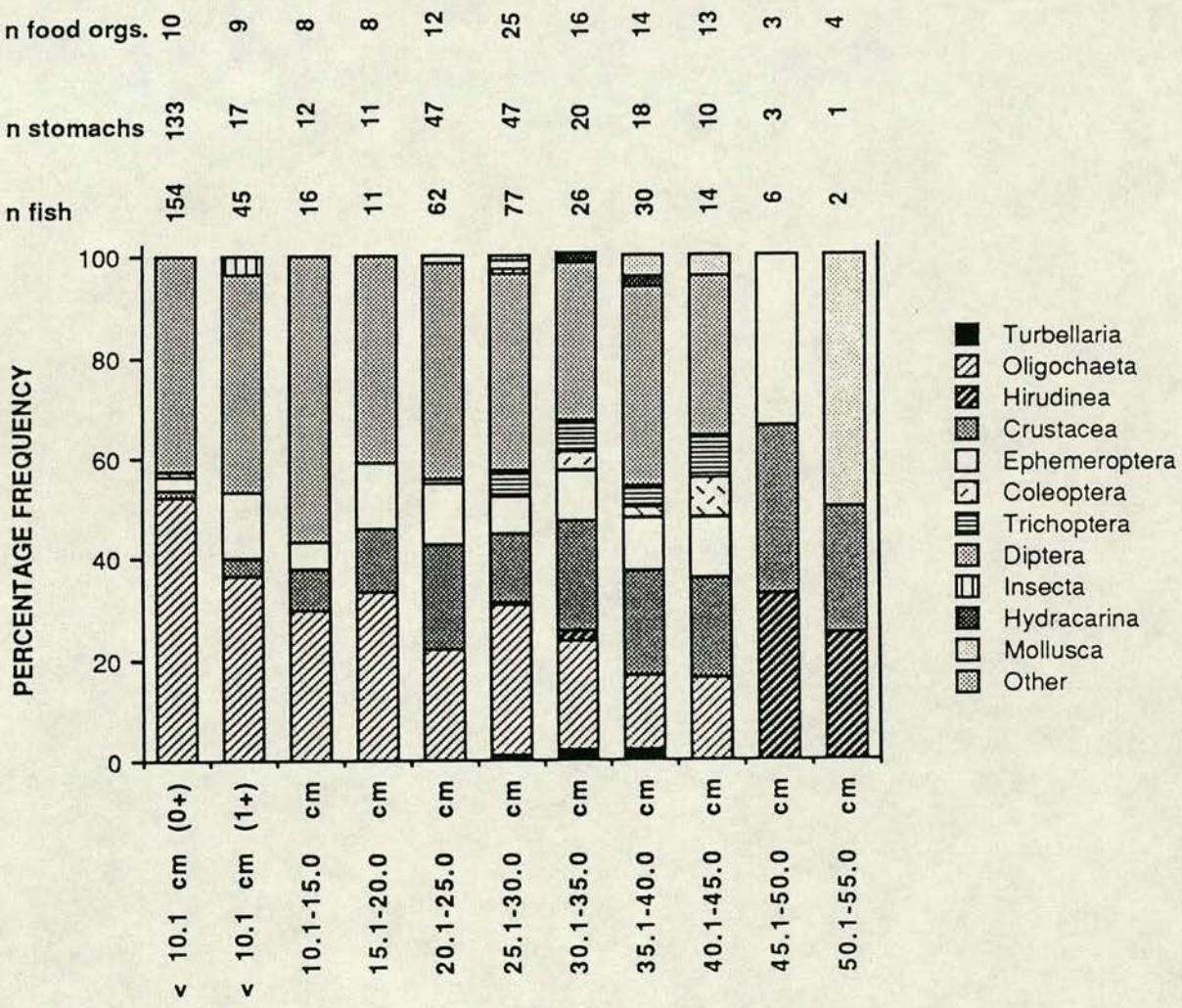


FIGURE 2.12 Width and length of the food organism, and interpupillary distance (IPD) in centimetres

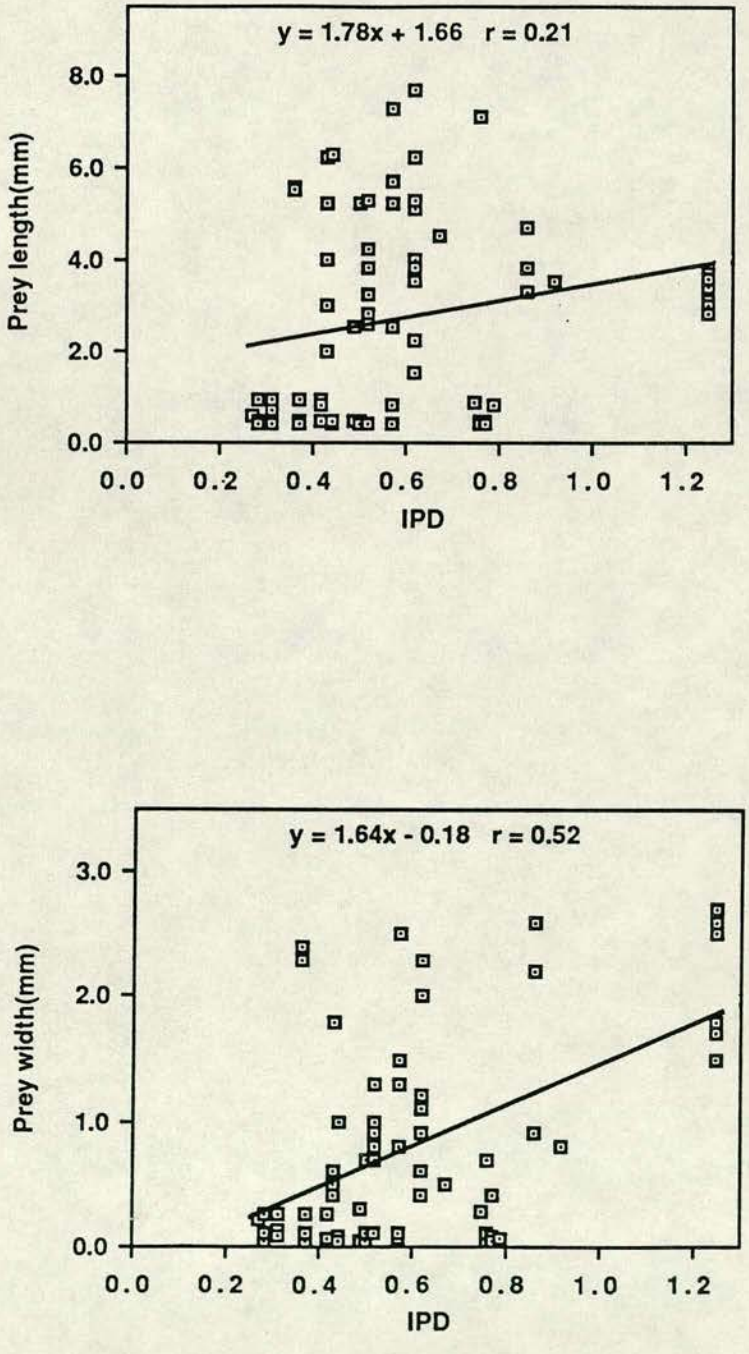
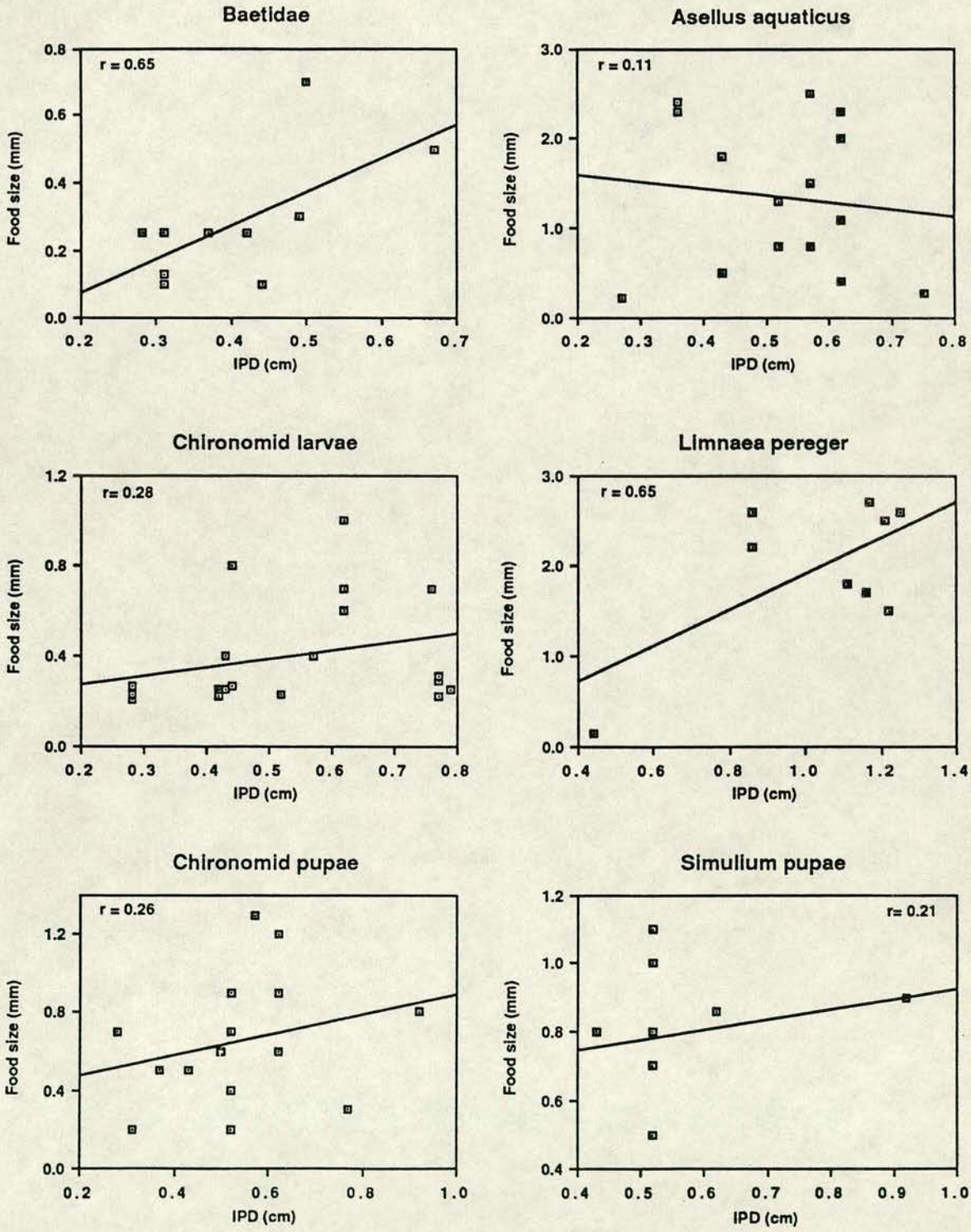


FIGURE 2.13 Width of individual food organisms and interpupillary distance (IPD)



The length and width of six of the 'common' food organisms were regressed against interpupillary distance (Figure 2.12). There was a significant relationship between the widest prey organism and interpupillary distance, $r = 0.52$; $p<0.001$, although smaller prey items were also taken by larger eels, as shown by the scatter of points in Figure 2.12. There was no significance in the length of prey taken by a particular size of eel, $r = 0.23$; $p>0.1$.

The width of individual organisms were regressed against interpupillary distance (Figure 2.13). The width of *Limnaea pereger* shells and Baetidae found in the stomach contents was significantly correlated with interpupillary distance, but there was no significance in an organism of similar size, that is, *Asellus aquaticus*. There was no significance in the width of chironomid larvae and pupae or *Simulium* sp. pupae.

2.5.4 DISCUSSION: The effect of different types of head shape on diet composition can be disregarded in the population of eels studied as eels were within the 'narrow-headed' range. The main food organisms were chironomid larvae and naid worms, which was in accord with other observations of 'narrow-headed' eels and Thurow's theory that head shape could be determined by prey size (Thurow, 1957). The counter argument of predisposition to a particular diet (Opuszynski, 1963) could not be tested, where the frequency and therefore choice of food items of different sizes was low.

The composition of the diet changed with size of eel. Some food organisms were consumed in higher proportions by certain size groups of eels, for example, smaller size groups took more Diptera and Oligochaeta, this was also observed by Hussein (1983) and Opuszynski (1963). Elvers were limited to 10 species, whereas the diet of eels in the intermediate size groups, that is, 20 cm - 35 cm, included more than 30 species. Diversity decreased with further increase in size group of eel. The eels in the intermediate size groups contained more plant matter and stones which may corroborate the less discriminate, 'shovelling' method of gathering food as the explanation for increased diversity of diet (Tesch, 1973). Change of diet with size of eel has been observed previously (Frost, 1946; Boëtius and Boëtius, 1967; Moriarty, 1973a; Sinha and Jones, 1975; Tesch, 1973 and Hussein, 1983). It has been observed that aquatic invertebrates decrease in importance in eels over 50 cm, which become increasingly piscivorous (Frost, 1946 and Moriarty, 1972b), but could not be explored in this study, as only 2 eels larger than 50.0 cm were sampled.

The largest width of food item taken was positively correlated with size of eel,

and some food species were restricted to certain size groups of eels, molluscs for example, were only taken by eels over 15 cm length, which has been explained by the necessity for stronger jaws (Tesch, 1973) rather than a purely size limiting factor. This indicates a degree of selection of food organisms.

2.6 FOOD SELECTION

This section examines the correspondence between the quantity of prey animals in the benthos, and the composition of the eel stomach contents in order to determine the degree of preference shown for a particular size or species of food item.

2.6.1 METHOD: It is assumed that the relative abundance of the fauna in the benthic samples is indicative of the availability of these food items to the eels. Selection is quantified by determination of the ratio of the food items in the stomach contents to the environment. The index of selection, the 'electivity index' developed by Ivlev (1961), and now in general usage (Windell and Bowen, 1978) was used. It is defined as follows:-

$$E = \frac{r_i - p_i}{r_i + p_i}$$

where E = electivity index

r_i = percentage composition by number of prey i in the stomach contents

p_i = percentage composition by number of prey i in the environment

The values of the electivity index range from -1 to +1, indicating complete rejection or complete positive selection respectively. However, this does not take into account many of the oligochaetes present in the stomach contents, but unquantifiable by number, because of the rapid effect of digestion. Therefore, a measure of the occurrence of each organism was used, by substitution of 'percentage composition by number' (N) in Ivlev's equation with ' percentage frequency of occurrence' (O).

Both types of electivity indices were used to determine the degree of selection of the major taxonomic groups of food organisms during each season at Sites 1 and 2, and are shown in Figures 2.14 and 2.15 respectively. Samples of bottom fauna and eels were taken at the same time, from similar areas, at monthly intervals.

2.6.2 RESULTS AND DISCUSSION: The major food organisms were positively selected when their availability in the benthos was lowest, that is, in summer for Oligochaeta and autumn and winter for Diptera. Other organisms were preferred when present in the benthos, including Crustacea, Trichoptera, Mollusca and Ephemeroptera. Coleoptera and Hirudinea were selected less, and Turbellaria were selected and rejected on different occassions, which identifies a limitation of Ivlev's index, where a chance occurrence can result in extreme positive or negative indices (Strauss, 1979). Hydracarina were not positively selected. Increased frequencies of *Asellus aquaticus*, *Simulium sp.* larvae, *Limnaea pereger* and species of Trichoptera resulted in higher frequencies in the stomach contents, whereas increased numbers of *Nais*, chironomid larvae and pupae, or Ephemeroptera, did not appear to affect intake. There was evidence of both selection of individual organisms and less discriminating methods of feeding. It appears that the eel is quite versatile in its feeding methods and is able to change from a diet consisting largely of oligochaetes, which would entail digging into the substrate, to one of chironomid larvae which were found amongst the vegetation and on the rock surfaces.

FIGURE 2.14 Electivity indices for major taxonomic groups at Site 1

(N = % composition by number, O = % composition by occurrence)

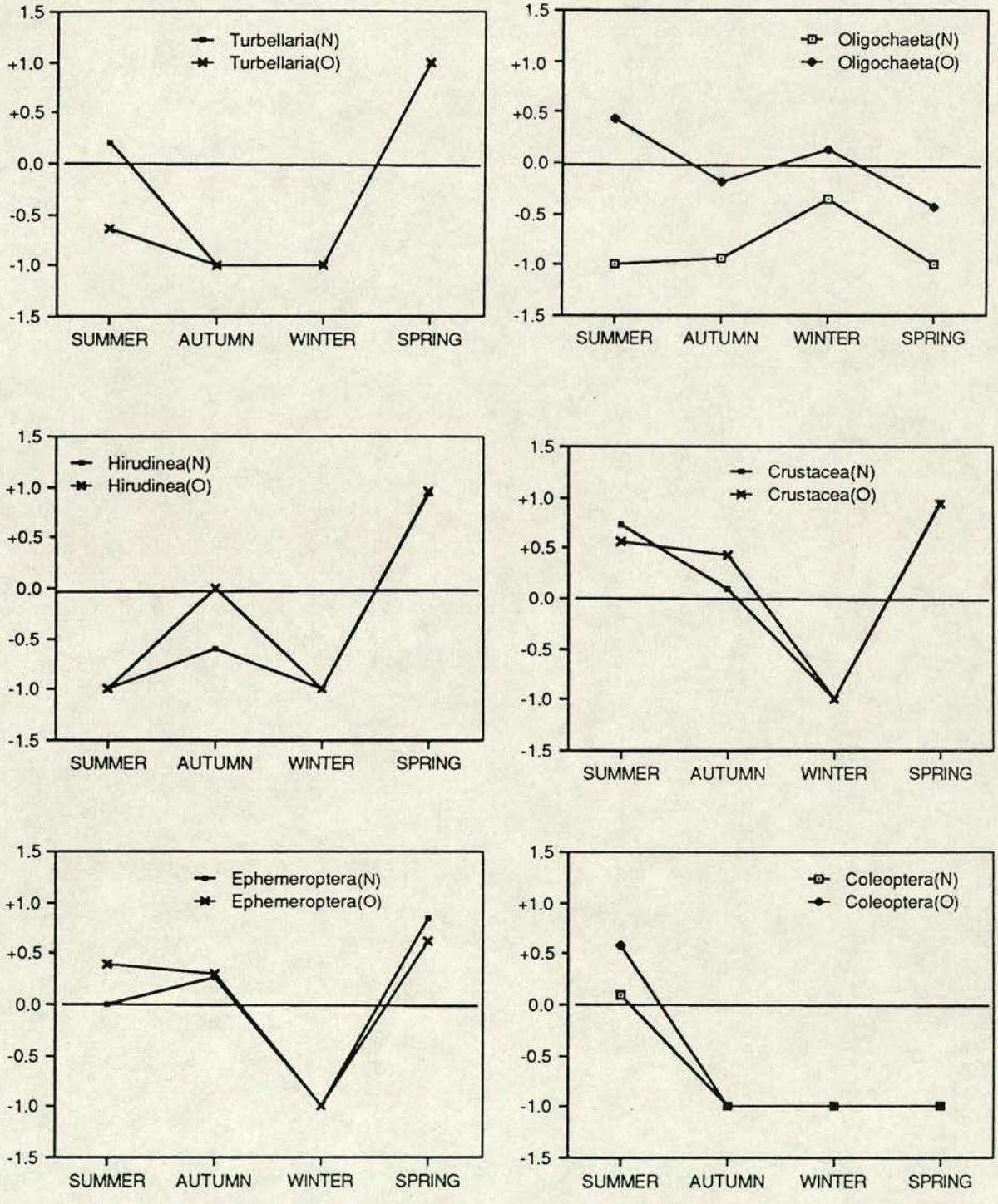


FIGURE 2.14 continued. Electivity indices for major taxonomic groups at Site 1 (N = % composition by number, O = % composition by occurrence)

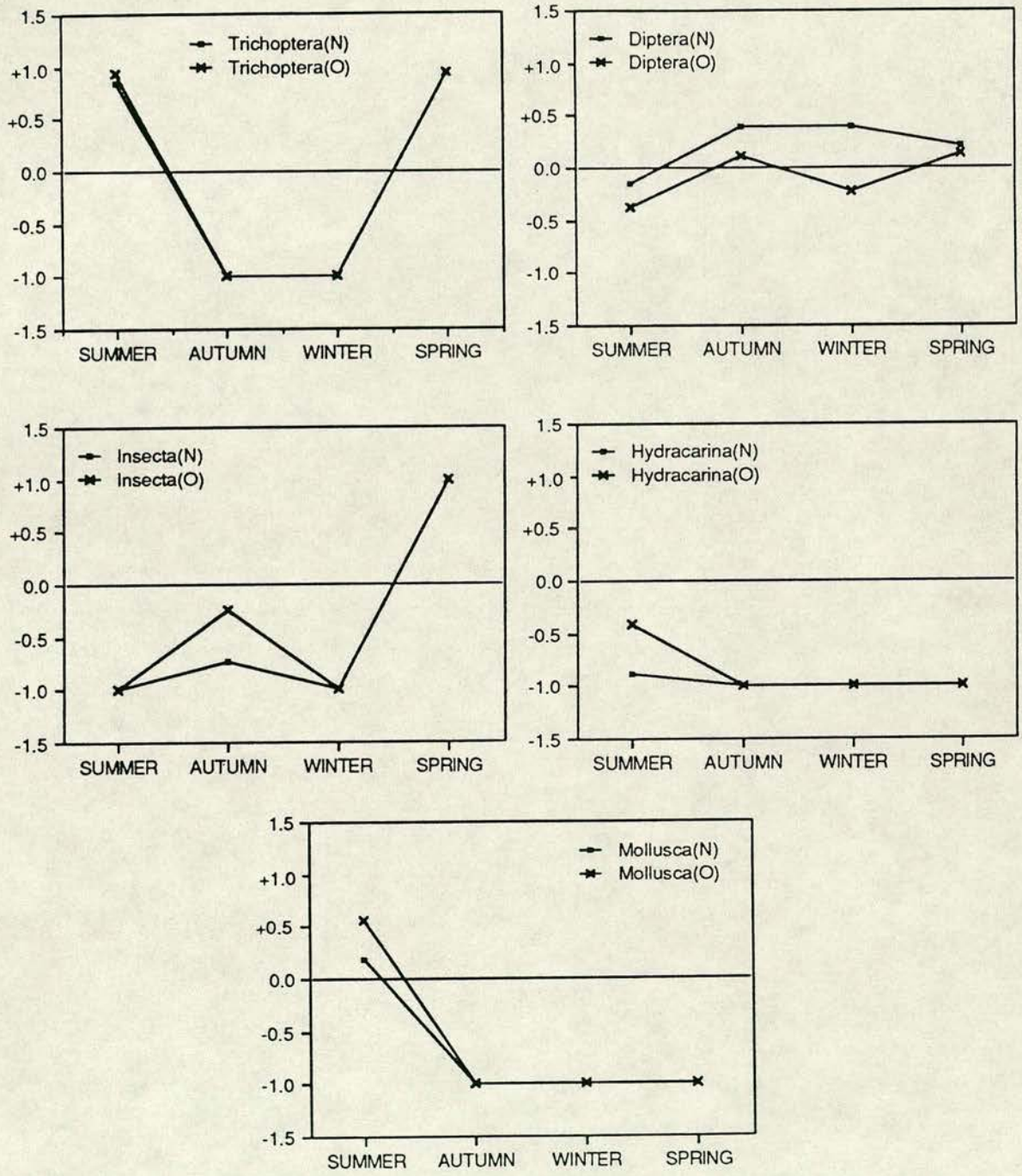
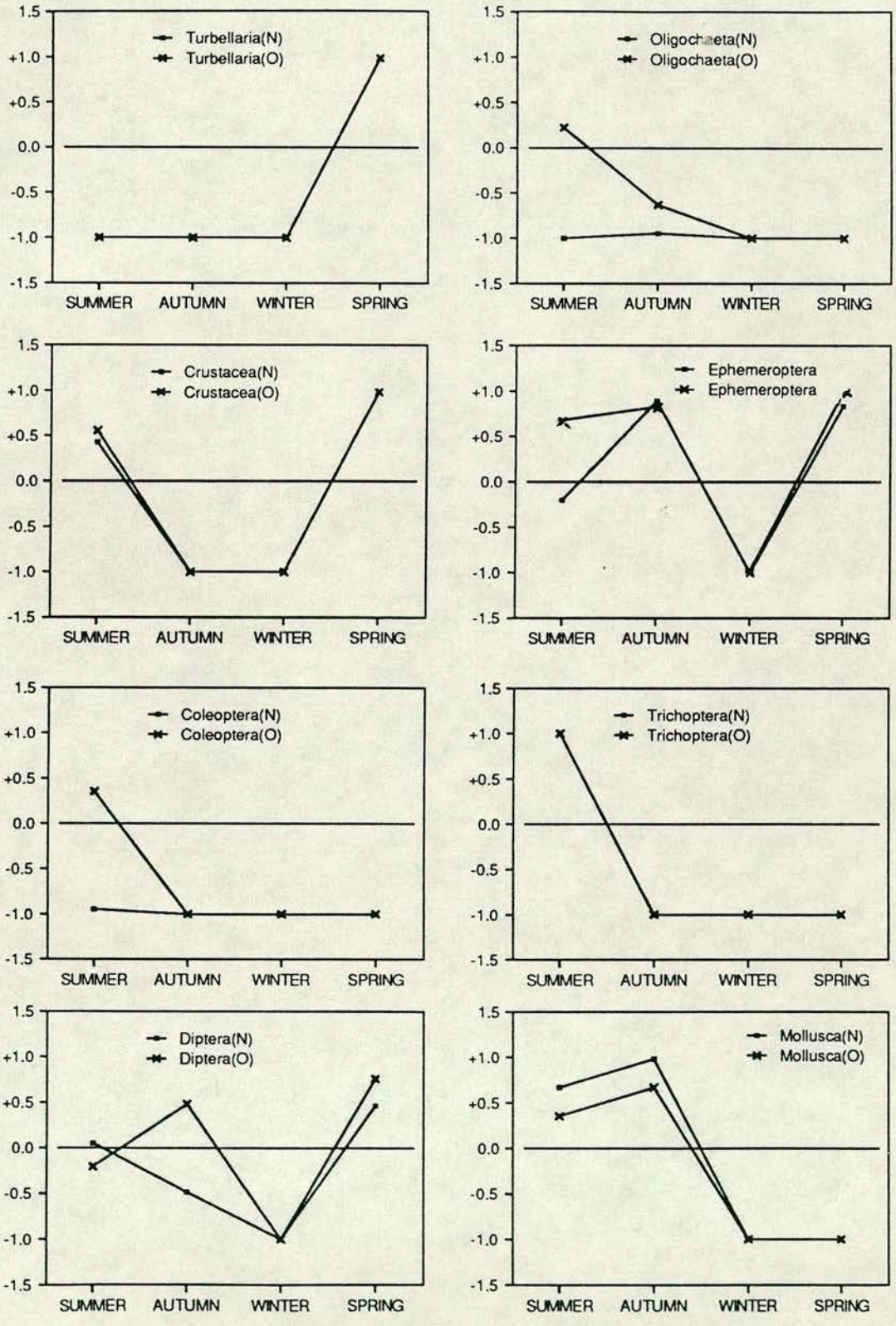


FIGURE 2.15 Electivity indices for major taxonomic groups at Site 2

(N = % composition by number, O = % composition by occurrence)



2.7 SUMMARY

1. The River Almond was subject to a continual input of pollutants and occasional shock pollution events which were mainly of an organic nature. The water was greatly enriched although stone loach *Neomacheilus barbatulus* and brown trout *Salmo trutta* were present, both pollution sensitive species of fish.
2. There was a high species diversity in the benthic fauna dominated by pollution tolerant species, such as, naid oligochaete worms and chironomid larvae, with lower frequencies of *Asellus aquaticus*.
3. The eels' diet included over 30 species of food organism but consisted mainly of naid worms and chironomid larvae with less *Asellus aquaticus* and species of Ephemeroptera.
4. Feeding activity was seasonal and closely related to water temperature above 10 °C, but may be intermittent in some eels which had empty stomachs during this time.
5. The River Almond eel population were 'narrow-headed'.
6. The composition of the diet reflected the relative frequencies of naid worms and chironomid larvae in the benthos, although the electivity for both was not high, and other organisms were preferred, that is, *Asellus aquaticus*, *Simulium* sp. *Limnaea pereger* and species of Trichoptera and Ephemeroptera.
7. Diet composition changed with size of eel, where species diversity was restricted in glass eels and elvers and was most diverse in the' intermediate' size range, that is, 20-35 cm. This was related to behaviour of the eel and the size of the food organism, where width was significantly correlated with interpupillary distance, which is proportional to mouth size.
8. The eel was quite versatile in its feeding behaviour where both selection of individual organisms and less discriminating gathering methods were used.

CHAPTER 3

AGE AND GROWTH

3.1 INTRODUCTION

Estimates of age and growth rate are fundamental to an understanding of the biology and population dynamics of fish. This chapter is concerned with age determination of eels in the River Almond, Scotland, and the intrinsic factors affecting growth including age, sexual maturity, sex and heredity. Other, more extrinsic factors affecting growth, including parasitism, disease, competition, predation, human intervention and other environmental factors are discussed in Chapter 4.

3.2 METHODS (The general sampling procedure and sampling methods are described in Chapter 1.2.1)

3.2.1 Sampling methods

284 eels and pigmented elvers from two sites on the River Almond were caught over a 13 month period. Glass eels were not considered to be established in the population until pigmentation, after which time they are described as elvers. Total length to the nearest 0.1 cm, and weight minus gut contents, to the nearest 0.01 g for eels 1 g and over, and 0.001 g for eels less than 1 g, were recorded and the otoliths extracted for age determination. Otoliths were removed by splitting the skull anterior-posteriorly along the mid-cranial suture. The brain was deflected and the sacculus plucked from the inner ear cavity with fine forceps. The sagitta was removed and cleaned in alcohol before dry storage in small, labelled envelopes. Sex was determined by macroscopic examination of the gonads.

3.2.2 Age determination

The most frequently used method of age determination in fish is the interpretation and counting of growth zones, or growth checks, which are deposited in calcified structures such as scales, opercula, vertebrae and otoliths. In the eel, growth rings are most clearly seen in the sagittal otoliths, which grow by accretion from the endolymph fluid which bathes them (Love, 1980). It is probable that estimates of age over the range of rapid growth are more accurate than when growth is reduced. This

underlies the requirement for validation of the accuracy, as distinct from reproduceability, of age estimates used (Beamish and McFarlane, 1983). These authors state that only by mark-recapture studies or use of fish of known-age can all age classes in a population be validated and suggest that if this is not possible, fish should be aged by several methods and the possibility of errors in age estimates considered. Validation of the ageing technique was not feasible in this study because of the short time available and the problems associated with mark-recapture studies on a migratory species such as the eel. However, two ageing methods of whole otoliths were used, that is, 'burning and cracking' (Christensen, 1964; Moriarty, 1973b) and 'clearing' (Völlestad, 1985). Measurements of otolith length to determine the body: otolith relationship were also taken (Ricker, 1968).

3.2.2.1 Method 1: Burning and cracking of otoliths (Christensen, 1964; Moriarty, 1973b).

1. The otolith is cracked through the nucleus (focus) before burning, by pressing a needle towards the cavern on its convex side. To prevent scattering of fractured pieces of otolith the cracking is done in a closed petri dish, through a hole in the lid.

2. The pieces are placed on a scalpel blade and kept in a bunsen flame for 30-60 seconds.

3. To prepare for viewing and storage, the pieces are embedded in a resin called 'Isopon' (manufactured by Automobile Plastics Co.Ltd., Hertfordshire, England). A microscope slide is coated with a film of 'Isopon' and marked-out into compartments with a needle. The pieces of otolith are picked-up with a mounted needle, tipped with the resin, which is adhesive enough to transfer them to the slide, where they are set at the required angle, in a particular compartment, before the resin hardens.

4. When the resin has hardened the whole slide is examined through water, under a binocular microscope at x 20 magnification, with reflected light.

5. A pattern of alternating broad, opaque (white) and narrow, translucent (black) rings, consisting of calcium carbonate and burnt organic material respectively, is seen and correspond to periods of growth and growth check. Each broad and narrow ring together is correlated with a yearly event, and is known as an annulus.

Several attempts have been made to test the validity of this method. Comparison of growth rates obtained from otoliths with growth rates from tagged and recaptured eels have shown the accuracy of the method is good (Moriarty, 1983). There may be problems with the interpretation of double or multiple rings and the over-estimation of the age of young eels (Champ, 1968; Moriarty and Steinmetz, 1979). The reproduceability of the method is acceptable (Moriarty, 1983; Völlestad,

1985) and is comparatively rapid, requiring no expensive equipment.

3.2.2.2 Method 2: Clearing of whole otoliths (Völlestad, 1985).

1. Otoliths are cleared in 96 % alcohol for 18-24 hours before viewing.

2. They are viewed under a stereoscopic microscope, using reflected light against a dark background, with 96 % alcohol as refracting medium (Aass, 1972 in Völlestad, 1985).

3. One translucent zone (= growth check) and one opaque zone (= growth) is deposited in the otoliths annually (Völlestad, 1985).

Attempts to validate this method have involved tetracycline injected and individually marked eels but have not been successful, as all tagged eels showed no sign of growth after tagging (Völlestad *et al.*, 1988). Tetracycline is deposited in newly formed bone tissue, which is detected by its fluorescence for several years after marking (Weber and Ridgeway, 1962). The reproduceability of the method is good, and is as good as the burning and cracking method (Völlestad, 1985). The method is rapid and inexpensive but may require the user to be more experienced in otolith interpretation than the burning and cracking method.

3.2.2.3 Measurement of otolith length:

Otolith length, to the nearest 0.01 mm was measured using an ocular micrometer. Otolith length is defined as being equivalent to twice the caudal radius (Rossi and Villani, 1980).

3.2.2.4 Interpretation of otoliths:

Age is recorded from the time the glass eels enter fresh water. The elver otolith has an inner zone of seawater rings within a thick opaque band (methods 1 and 2), which represents the first year's growth phase in fresh water, bounded by the first winter's dark band (method 1)/ translucent band (method 2). These two complete rings represent the first year in fresh water (Sinha and Jones, 1967a). The number of rings on the otolith were used when allocating age groups to elvers (Sinha and Jones, 1967a) rather than assuming a general date for entry into freshwater (Frost, 1945).

3.2.3 Growth determination:

A measure of the growth of the eel population was made by using the mean size of each age class. Despite the inequality of growth rate between individuals, which is particularly wide in the eel (Deelder, 1957), the range of growth at each age in a particular habitat is confined within reasonable limits (Pitcher and Hart, 1982). Growth curves showing the degree of variation at each age class for the different sexes, and undifferentiated or immature eels, were drawn. Comparisons of growth were restricted to absolute or relative length and weight increments, because conventional growth patterns are not necessarily followed by eels. The largest eels reach maturity, which is known as 'silvering', and leave the system at different ages depending upon their growth rate. Thus, the exponential phase of growth may be interrupted by sudden maturation and emigration, rather than a physiological limitation causing a gradual reduction of growth rate the closer the maximum size is reached, such as is assumed in many growth models. A model frequently used in fisheries work is the von Bertalanffy (1957) and attempts to estimate parameters, or the parameters estimated, can be meaningless, for example, $L\infty$, the maximum theoretical length, was estimated at 900 cm (Sparre, 1979). Furthermore, the weight / length relationship, assumed to be $W = a L^b$ does not fit for eels as W/L^3 is not a constant throughout the growth period (where W = weight, L = length, b = an exponent and a = a constant) (Sparre, 1979). Both parametric and non-parametric growth statistics are shown, for comparison with other studies, although the populations in the River Almond were not normally distributed. The significance of differences between the samples with respect to length, weight and age were tested by means of Mann-Whitney *U* - tests (Siegel, 1956).

3.3 RESULTS

3.3.1 Otolith rings

The age recorded by both methods agreed in 79 % of the otoliths examined, differed by ± 1 year in 12 % and more than ± 1 year in 2 %. Otoliths were unreadable in 3 % and 1 % by the burning and cracking and the clearing methods respectively, and 3 % were unreadable by either method. This represents a reasonable degree of reproduceability when compared with other studies using these methods (Moriarty, 1983; Völlestad, 1985).

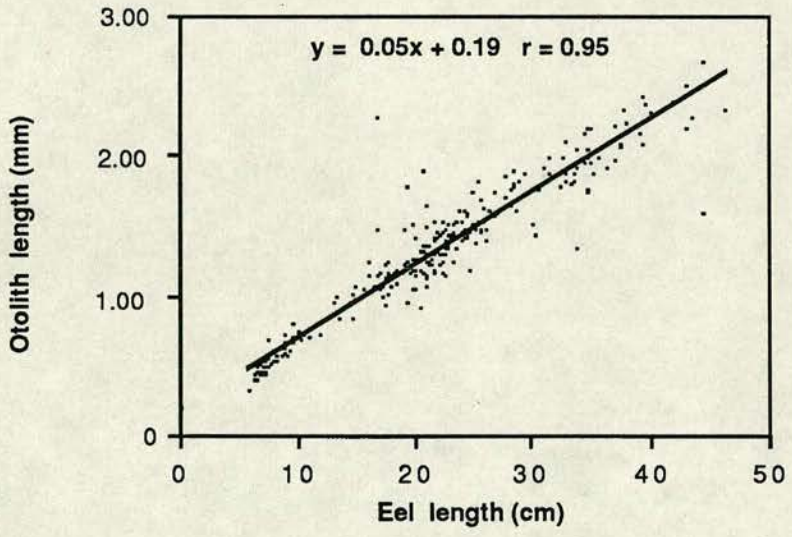
Otoliths showing a clear annual ring pattern, where the discrepancy between the two methods was ± 1 year were used in the growth studies. The age as read by the burning and cracking method was recorded where there was a discrepancy between the

two methods. Three eels with cauliflower disease, which may have affected growth, were discarded (Chapter 4.3). The eels from Sites 1 and 2 and each sex were sorted into age groups which ranged from year 0+ to year 11+. The length groups chosen were used consistently throughout the study.

3.3.2 Otolith size

There was a linear or isometric relationship between otolith length and eel length as shown in Figure 3.1, $r = 0.95$; $p<0.0001$.

Figure 3.1 Relationship between otolith size and eel length



3.3.3 Population structure

There was a large overlap in the size of individuals in adjacent age classes which necessitated the examination of age and length independently of each other, as shown in Tables 3.1 and 3.2 for Sites 1 and 2 respectively.

Table 3.1 Statistics of length and weight of age classes at Site 1

(n=sample size, Std. error = standard error)

Immature										Female		
Age class	0	1	2	3	4	5	6	7	8	5	6	7
n	28	10	25	46	39	14	10	10	2	1	4	5
Mean length(cm)	7.5	10.6	18.6	20.6	22.0	24.6	29.1	28.9	31.3	28.1	33.5	34.7
Std. error	0.23	0.87	0.58	0.33	0.51	0.93	1.62	1.37	2.65		3.84	2.16
Range in length	6.0-11.0	8.1-16.3	13.0-22.7	15.1-25.8	15.8-33.7	19.4-33.5	22.6-35.1	22.0-34.6	28.6-33.9		27.6-44.5	28.5-40.0
Mean weight(g)	0.446	1.615	8.99	11.91	14.53	22.29	43.40	43.19	49.28	34.41	71.26	52.90
Std.error	0.075	0.472	0.84	0.65	1.28	3.58	7.94	7.23	15.68		41.05	12.84
Range in weight	0.201-1.800	0.424-4.770	2.48-16.70	4.89-26.51	6.07-53.65	7.8-61.60	14.66-83.67	15.95-86.29	33.60-64.95		22.60-194.11	27.21-99.30
Median length (cm)	7.2	9.4	18.2	20.9	21.9	24.3	29.1	29.9	31.3		30.9	33.10
Median weight (g)	0.307	0.891	8.37	11.34	13.88	21.57	40.94	42.48	49.28		34.16	51.84

TABLE 3.2 Statistics of length and weight of age classes at Site 2

(n=sample size, Std. error = standard error)

Immature

Age class	0	1	2	3	4	5	6	7
n	10	8	5	3	4	6	1	3
Mean length(cm)	7.3	9.5	17.7	16.6	23.4	27.5	27.7	33.7
Std. error	0.25	0.66	1.44	2.39	0.49	1.69		2.65
Range in length	6.4-8.8	7.8-13.5	14.7-22.2	14.0-21.4	22.8-24.5	24.4-26.8		28.6-37.5
Mean weight(g)	0.365	0.967	9.04	7.41	19.13	35.51	36.37	64.25
Std. error	0.048	0.314	2.55	2.88	1.28	8.75		8.43
Range in weight	0.183-0.679	0.288-2.900	4.30-18.05	4.05-13.13	15.85-21.56	22.16-78.47		48.79-77.81
Median length (cm)	7.2	9.0	16.7	18.0	23.9	26.2		35.0
Median weight (g)	0.368	0.619	7.52	7.89	20.72	30.70		34.8

Female

Age class	4	5	6	7	8	9	10	11
n	1	3	8	6	6	3	1	1
Mean length(cm)	25.3	31.9	35.6	36.3	37.2	43.1	43.2	41.9
Std. error		2.24	1.29	2.04	1.71	1.98		
Range in length		28.7-36.2	33.6-39.0	31.5-43.1	33.3-44.5	39.5-46.3		
Mean weight(g)	24.68	51.74	75.76	82.05	89.77	135.88	149.21	128.22
Std.error		8.6	11.3	15.36	19.05	20.46		
Range in weight		42.51-68.93	46.78-118.30	49.19-137.11	62.46-182.34	100.96-171.79		
Median length (cm)		30.7	37.0	34.8	35.8	43.5		
Median weight (g)		43.79	73.74	78.31	68.63	134.60		

3.3.3.1 Age distribution

The percentage frequencies of age classes at both sites are shown in Figure 3.2. There were a greater percentage of young eels at Site 1, where 59 % were under year 5+ compared with 38 % at Site 2, although the frequency of year 1+ eels at Site 1 was lower, that is, 3.1 % compared with 11.4 % at Site 2. Eels at Site 1 were significantly younger (median = 3 years) than Site 2 (median = 5 years); $p<0.05$. The age range was greater at Site 2 where eels up to year 11+ were caught, compared with year 8+ at Site 1.

3.3.3.2 Length distribution

The percentage frequencies of the different length groups are shown in Figure 3.3 where it should be noted that eels less than 10.1 cm were sub-divided into year 0+ and year 1+. The frequency of smaller eels was greater at Site 1, where 80 % were below 25.1 cm, compared with 47 % at Site 2. Eels at Site 1 were significantly shorter (median = 20.8 cm) than those from Site 2 (median = 25.8 cm); $p< 0.001$.

Monthly length group frequencies at Site 1 and Site 2 are shown in Figures 3.4. and 3.5 respectively. Pigmented elvers (year 0+) did not appear in the catch until September at Site 1 and October at Site 2. The 15.1 - 20.0 cm and 20.1 - 25.0 cm groups were strong throughout the year at Site 1. Eels were caught in every month at Site 1, but were not present in November, December and March at Site 2.

3.3.3.2 Weight distribution

Eels were significantly heavier at Site 2 (median = 25.20 g) than Site 1 (median = 11.57 g); $p<0.001$.

3.3.3.3 Population density

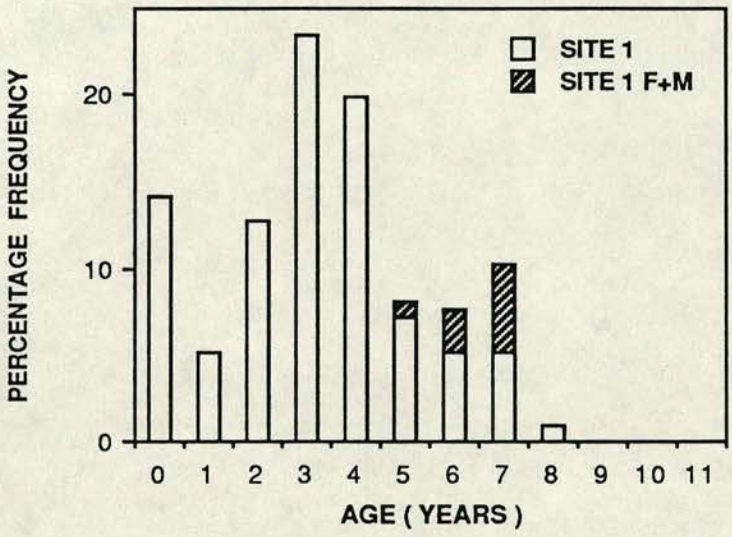
The sampling method did not give a true estimate of population density but was probably an indication of lowest density (Chapter 1.2.1.2). The number of eels per m^2 was 0.14 and 0.05 at Sites 1 and 2 respectively, and biomass was 2.74 g and 2.31 g per m^2 at Sites 1 and 2 respectively.

3.3.3.4 Distribution of maturing eels

Maturing eels were present in the samples from April to September at Site 1 and May to October at Site 2, only two of which were male, and from Site 1, as shown in Figures 3.4 and 3.5. The frequency of sexually differentiated eels was higher at Site 2, where 38 % of the eels caught were female compared with 5 % female and 1 % male at Site 1. Earliest differentiation occurred from year 4+, 25.1 cm and 22.60 g, the values

being independent of each other. Female eels were longer than immature eels of the same age as shown in the length growth curves in Figure 3.6 and heavier also, as shown in the weight growth curves in Figure 3.7. There were no significant differences between immature and maturing female eels of the same age, at each site, and between sites, with respect to length and weight, except for year 7+ females at Site 2. These were significantly longer (median = 34.8 cm) than Site 1 (median = 29.9 cm); $p<0.05$, and heavier at Site 2 (78.31 g) than Site 1 (42.48 g); $p<0.05$. There was less overlap in length than age or weight between immature and maturing eels, which suggests that sexual maturation is related to length (Kuhlmann, 1974).

FIGURE 3.2 Percentage frequency of age classes at Site 1 and Site 2 (□=undifferentiated, F=female, M=male)



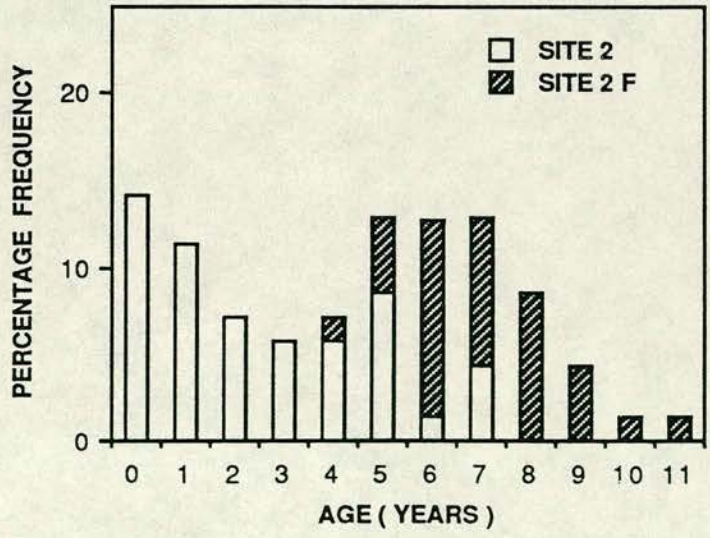


FIGURE 3.3 Percentage frequency of length groups at Sites 1 and 2
(□=undifferentiated, F=female, M=male)

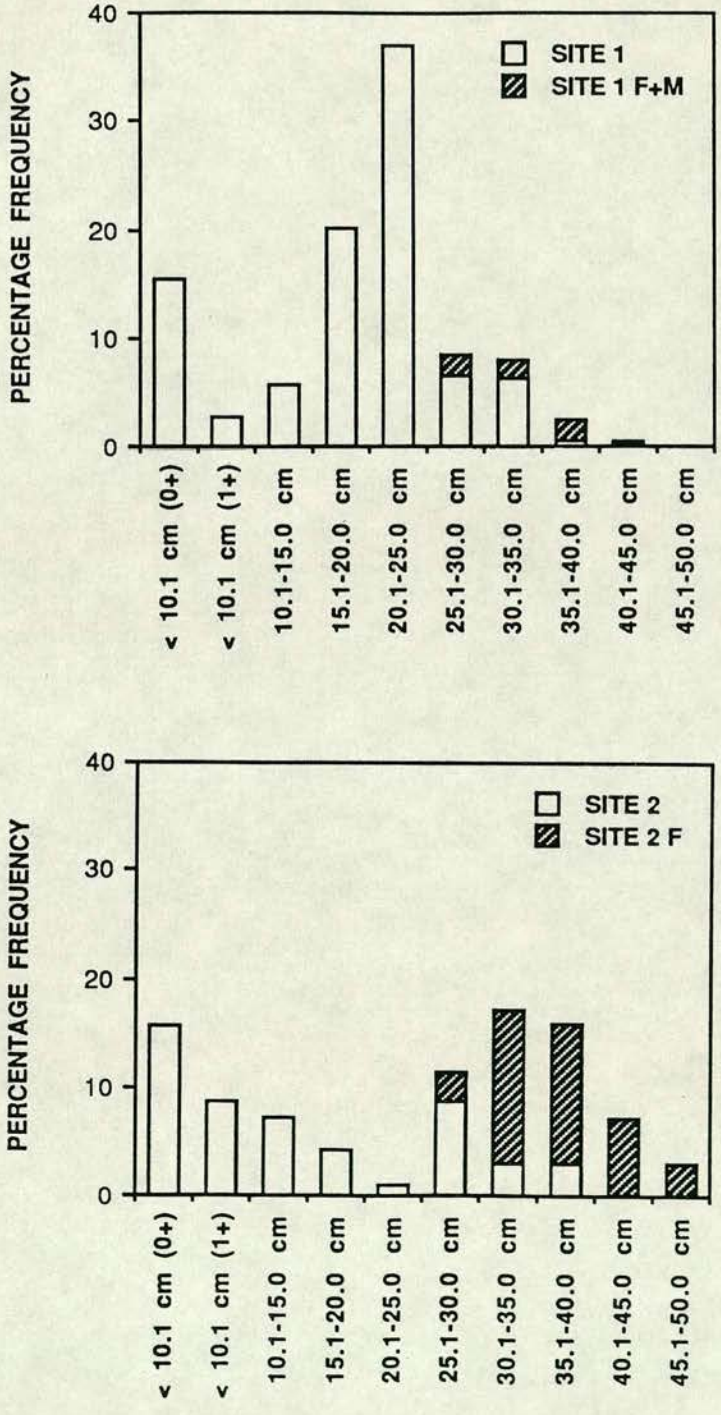


FIGURE 3.4 Percentage frequency of length groups in each month at Site 1 (□ =undifferentiated, F=female, M=male)

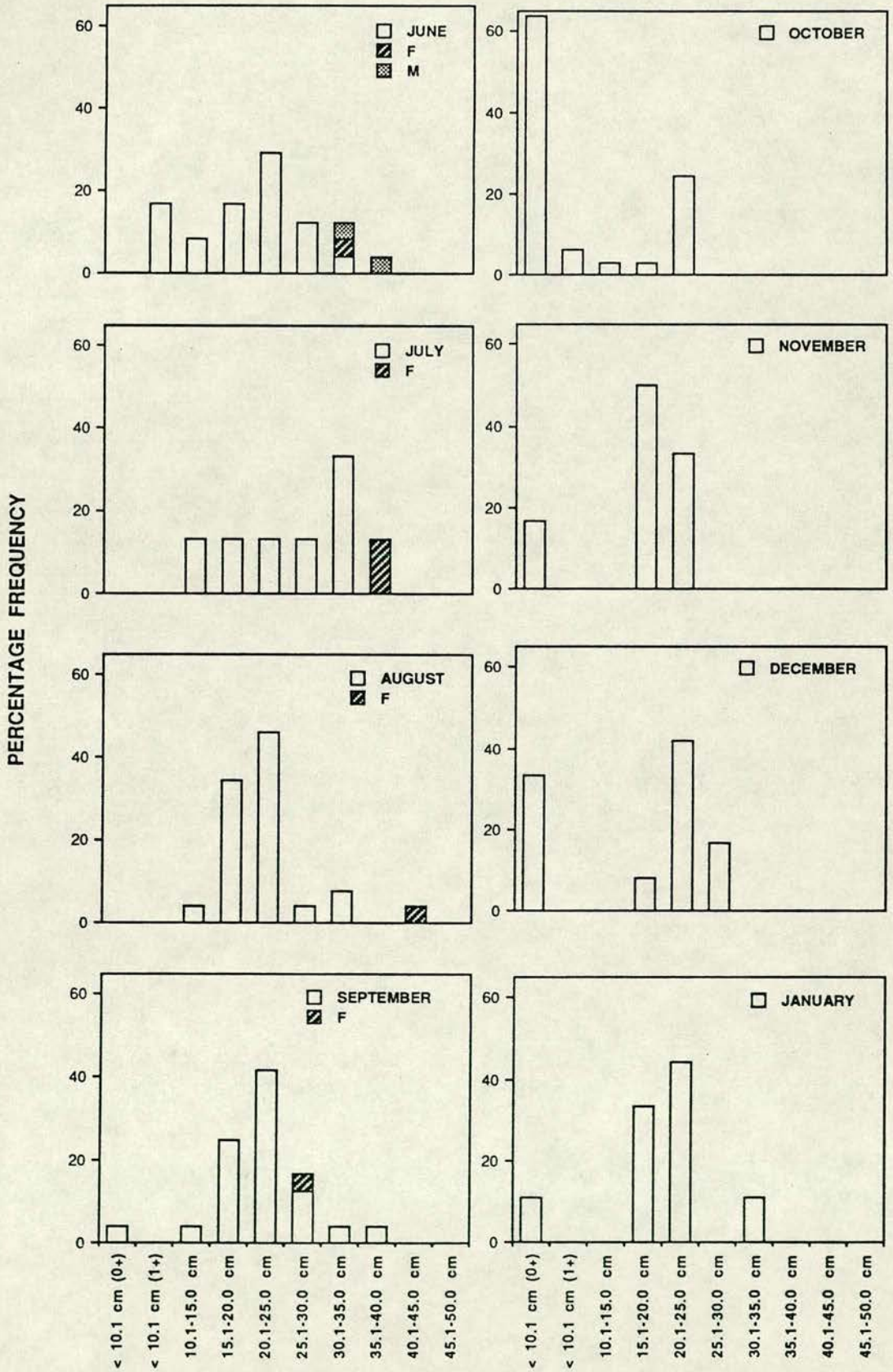


FIGURE 3.4 continued. Percentage frequency of length groups in each month at Site 1 (□ =undifferentiated, F=female, M=male)

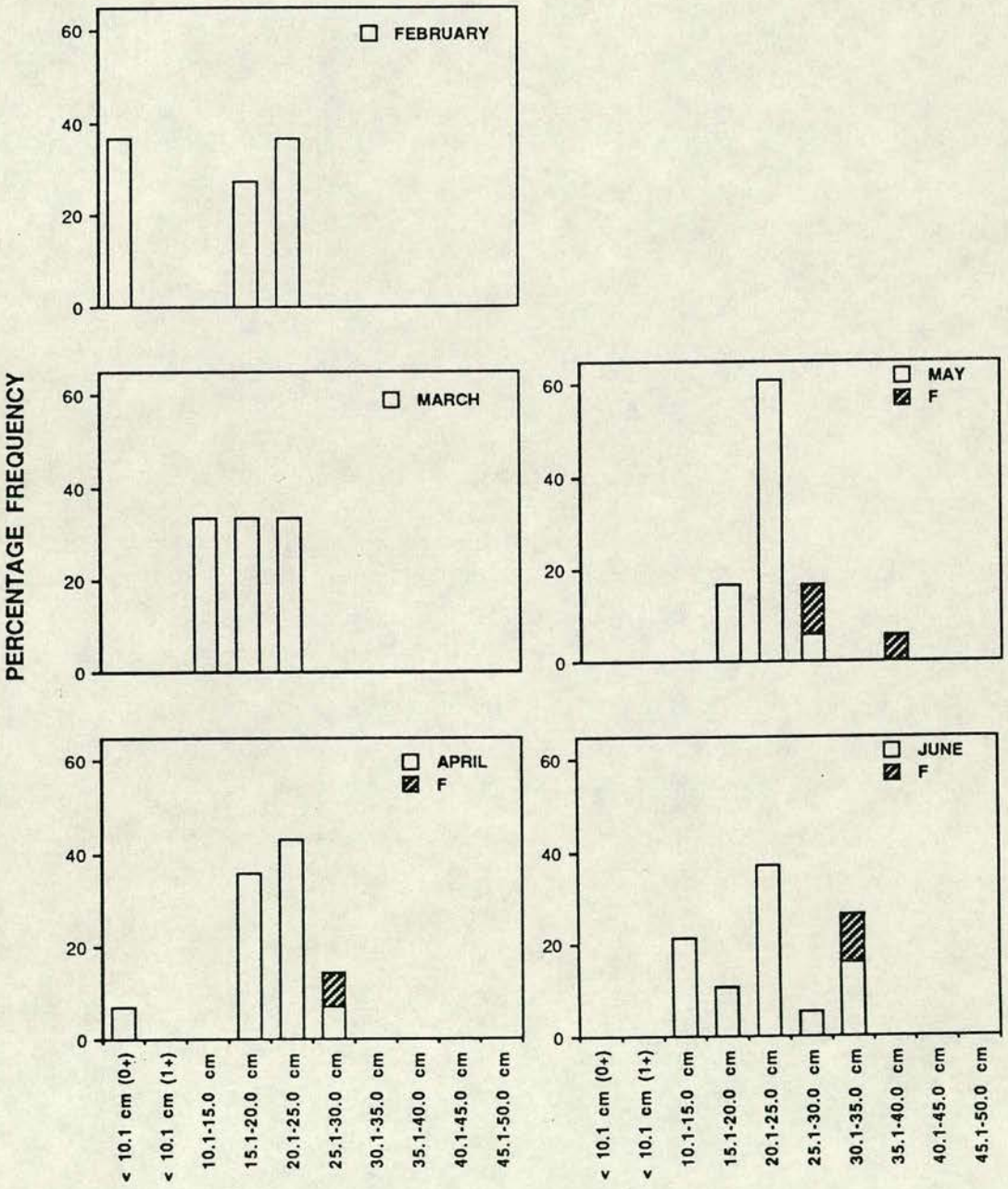


FIGURE 3.5 Percentage frequency of length groups in each month at Site 2 (□ =undifferentiated, F=female, M=male)

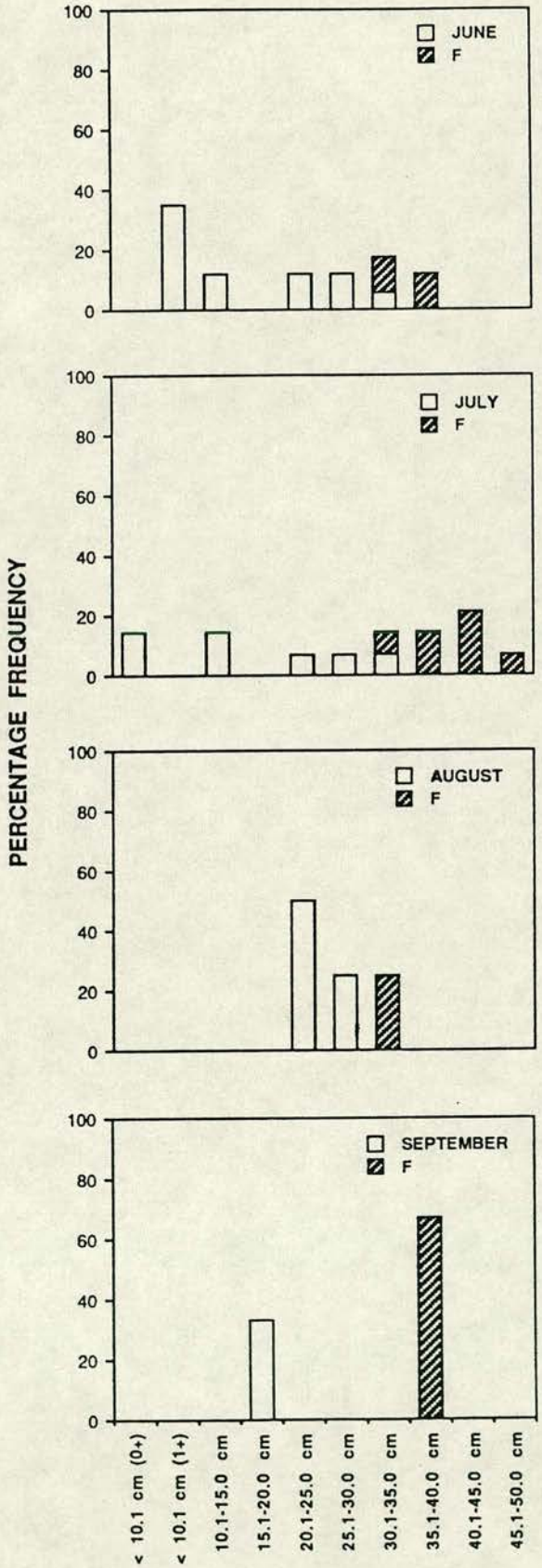
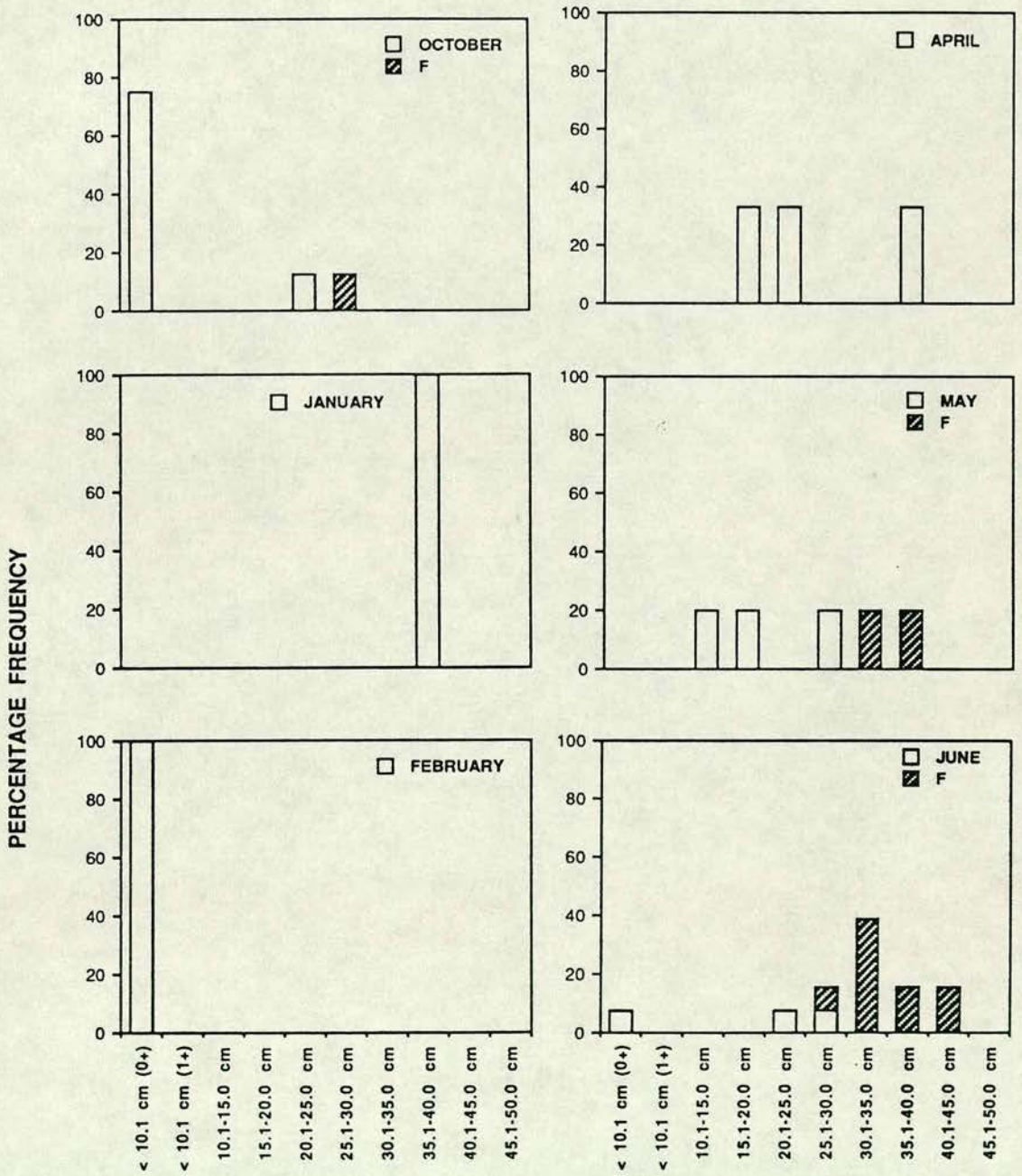


FIGURE 3.5 continued. Percentage frequency of length groups in each month at Site 2 (□ =undifferentiated, F=female, M=male)



3.3.4 Growth

3.3.4.1 Growth in length

Length growth curves for eels at both sites are shown in Figure 3.6. There was no significant difference in the growth in length of immature year classes between sites, that is, years 0+, 1+, 2+ and 3+; $p>0.05$. Growth rate of each year class at both sites, expressed as absolute length increments in centimetres per year, is shown in Table 3.3. Mean annual incremental growth in length of the immature populations at Sites 1 and 2 was 3.0 cm and 3.8 cm respectively.

3.3.4.2 Growth in weight

Weight growth curves for eels at both sites are shown in Figure 3.7. Growth in weight was more variable than length. Weight did not differ significantly between immature eels of the same age class at the two sites, except for year 4+, which were significantly heavier at Site 2 (median = 20.72 g) than Site 1 (median = 13.88 g); $p<0.05$ (Table 3.3). Annual incremental growth in weight of the populations at Sites 1 and 2 was 6.11 g and 9.13 g respectively.

Table 3.3 Annual length and weight increments of eels at Site 1 and Site 2 (l=length (cm), w=weight (g))

	Site 1				Site 2			
Age	**Immature**		**Female**		**Immature**		**Female**	
	l	**w**	**l**	**w**	**l**	**w**	**l**	**w**
1	3.1	1.17	-	-	2.2	0.60	-	-
2	8.0	7.38	-	-	8.2	8.07	-	-
3	2.0	2.92	-	-	-1.1	-1.63	-	-
4	1.4	2.62	-	-	6.8	11.72	-	-
5	2.6	7.76	5.4	36.85	4.1	16.38	6.6	27.06
6	4.5	21.11	1.2	-18.36	0.2	0.86	3.7	24.02
7	-0.2	-0.21	-	-	6.0	27.88	0.7	6.29
8	2.4	6.09	-	-	-	-	0.9	7.72
9	-	-	-	-	-	-	5.9	46.11
10	-	-	-	-	-	-	0.1	13.33
11	-	-	-	-	-	-	-1.3	-20.99

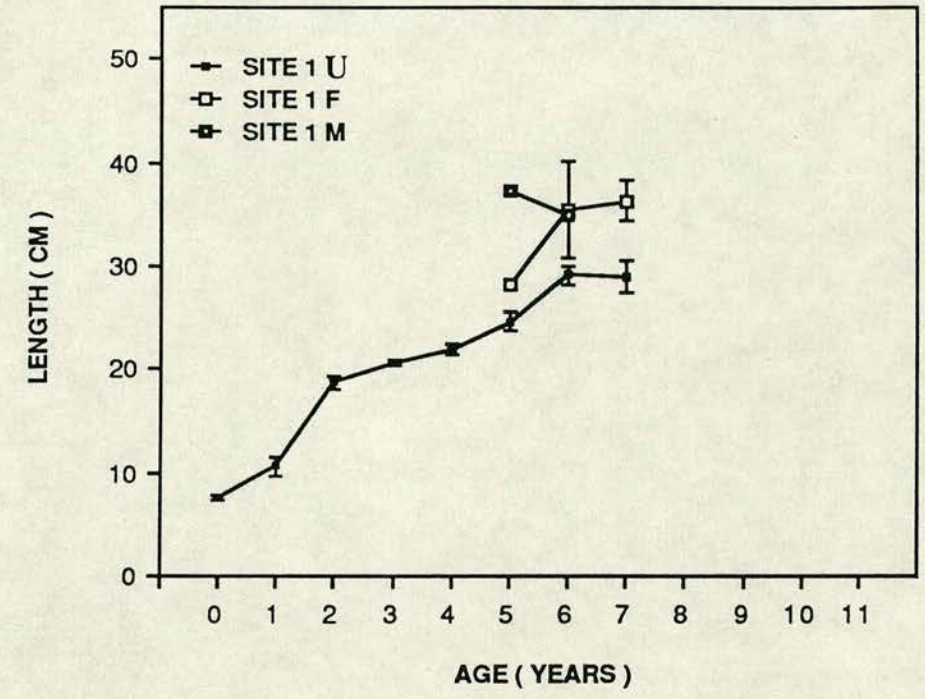
There was little difference in growth of each year class between the sites up to year 3+ and growth of year 2+ at both sites was extremely high, whereas growth of year 3+ was low at both sites, and 4+ were slow growing at Site 1 but not at Site 2. The females at both sites had a faster growth rate than the immature eels of the same age, except for year 7+ at Site 2.

3.3.4.3 Growth variation

Extremely marked differential growth within an eel age class was apparent in the River Almond population. (Tables 3.1 and 3.2). Variation was increased further by sampling of age classes through the year, where no allowance was made for progress through the growing season (Barbour, 1984). Further variation was caused by sex dependent growth, where the growth of females was faster than immature eels of the same age (Figures 3.6 and 3.7). The coefficient of variation (CV), where CV = 100 x standard deviation / mean, was calculated for immature and female eels in each age class, at both sites and is shown in Table 3.4. CV was greater for weight than length. Variation was higher in the younger age classes and lowest in the 3+ and 4+ year classes at Site 1, where growth of these particular year classes was slow.

FIGURE 3.6 Length growth curves at Site 1 and Site 2

(U=undifferentiated, F=female, M=male, bars indicate standard error of the mean)



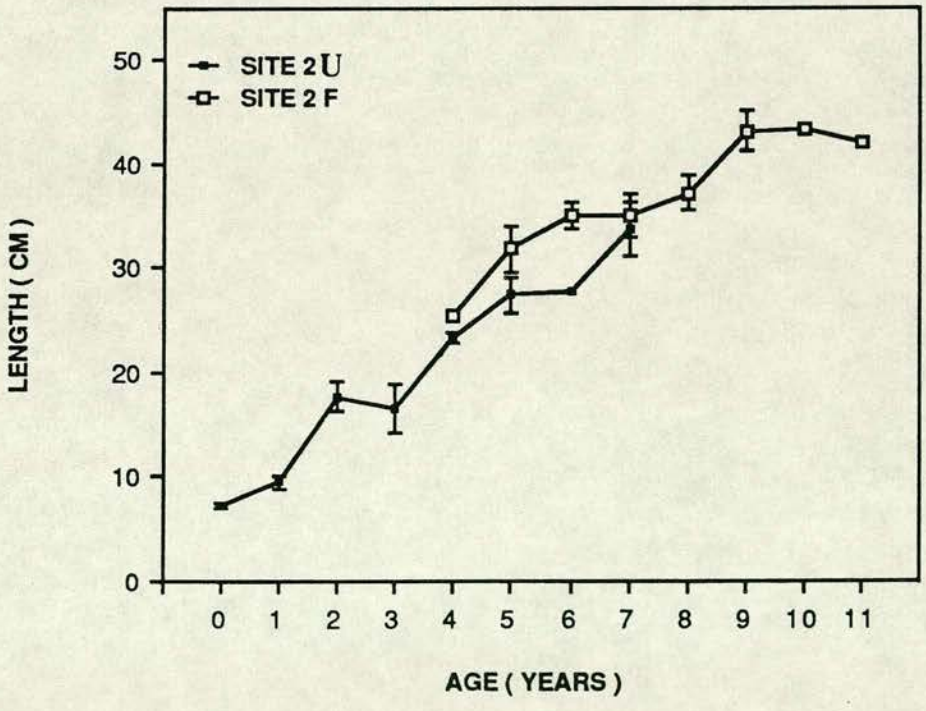
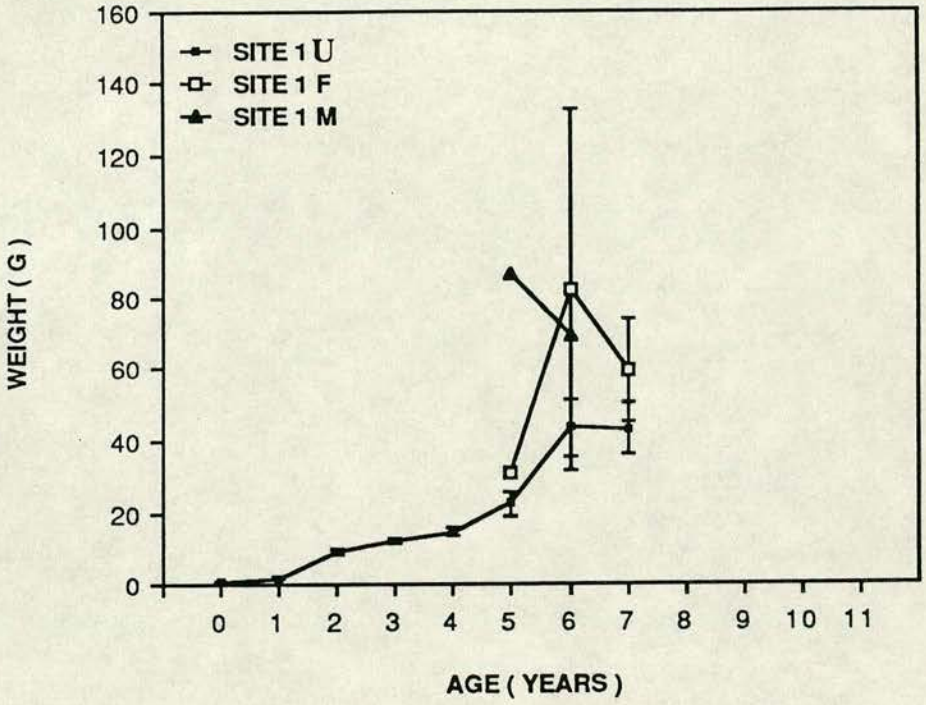


FIGURE 3.7 Weight growth curves at Site 1 and Site 2

(U=undifferentiated, F=female, M=male, bars indicate standard error of the mean)



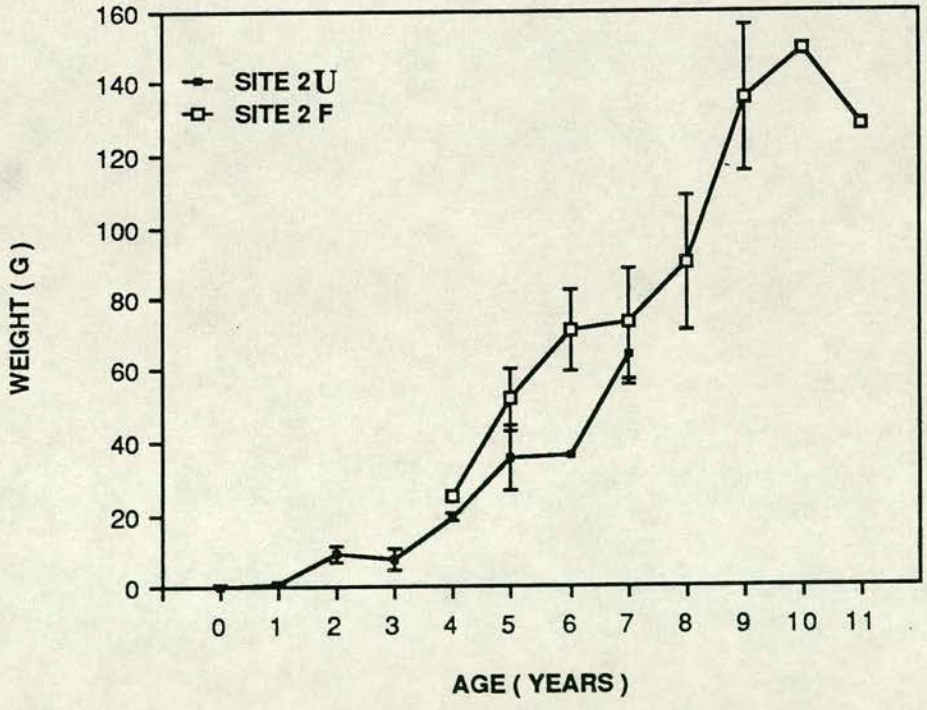


TABLE 3.4 Coefficient of variation of length and weight

Age class	Coefficient of variation			
	Immature		Female	
	Length	Weight	Length	Weight
SITE 1				
0	16.2	89.0	-	-
1	26.0	92.4	-	-
2	15.6	46.7	-	-
3	10.9	37.0	-	-
4	14.5	55.0	-	-
5	14.2	60.1	-	-
6	17.6	57.9	22.9	115.2
7	15.0	53.0	13.9	54.3
8	12.0	45.0	-	-
SITE 2				
0	10.8	41.6	-	-
1	19.7	91.8	-	-
2	18.2	63.1	-	-
3	24.9	67.3	-	-
4	4.2	13.4	-	-
5	15.1	60.4	12.2	28.8
6	-	-	10.3	42.2
7	13.6	22.7	13.8	45.9
8	-	-	11.3	52.0
9	-	-	8.0	26.1

3.4 DISCUSSION

There were a greater number of younger and smaller eels at Site 1, that is, 0.14 and 0.05 eels per m^2 and a biomass of 2.74 and 2.31 g per m^2 at Site 1 and Site 2 respectively which compares with 0.06 eels and 3.36 g per m^2 in the River Tweed, Scotland (Hussein, 1983). The structure of the eel population at both sites differed and was probably due to the weir between Sites 1 and 2, which acted as a partial barrier to up-river migration. A restricted home range is likely to affect population age structure (Tesch, 1973). It is commonly found that the number of eels in a river system decline with distance from the sea, which is related to the time required for migration, where water velocity is considered a major factor (Moriarty, 1972b). Migration rates of 20 km per year were found in the River Severn (Aprahamian, 1988) although 45 km per year are possible in the River Tweed (Hussein, 1983).

Mean incremental growth of the population at both sites, that is, 3.0 cm and 3.8 cm at Sites 1 and 2 respectively, was in the same order as other temperate water studies, that is, 4.0 cm (Frost, 1945), 3.5-4.0 cm (Sinha and Jones, 1967) and 2.2-3.2 cm (Moriarty, 1983). Growth was similar at both sites until year 4+, after which eels were significantly heavier at Site 2 than Site 1. Incremental growth of the year 2+ age class was extremely high at both sites, that is, 8.0 cm and 8.2 cm at Sites 1 and 2 respectively, whereas growth of year 3+ and 4+ eels at Site 1 was low, that is, 2.0 and 1.4 cm respectively.

It has been reported that competition for food is most acute amongst the smaller size groups (Sinha and Jones, 1967a), these authors found poor growth of eels up to year 2+ in the Welsh River Rhyd-hir. In the River Almond the benthic fauna consisted largely of naid worms and chironomid larvae, which were the main food organisms of the smaller groups of eels, that is, years 0+,1+ and 2+. This diet may be particularly suitable and allow a high efficiency, which might explain the very high growth rates of the 2+ age class. (Chapter 2). There may have been competition for the larger food items, that is, mayflies, crustacea and stoneflies amongst the large numbers of eels in years 3+ and 4+, who were restricted to an energy demanding collection of many small items at a period in their development when metabolic rate, per unit body weight, is high (Weatherley, 1972). They show flexibility in growth rate as a means of population control, that is, competition dependent growth. As the eels grow the number of types of different food organisms increases (Figure 2.11) and competition for larger food items is probably reduced, allowing a faster growth rate from year 5+. The cannibalistic nature of larger eels has been cited as a possible reason for their increased growth rate (Sinha and Jones, 1967a), but cannibalism was evident on only

two occasions in this study (Chapter 2.4.2).

The growth rate of sexually differentiated eels was faster than immature eels of the same age, although not all fast growing eels were sexually differentiated. Only two males were caught, both at Site 1. An ecological distribution of both sexes has been reported, where higher salinity in coastal areas or river mouths favours male eels whilst females tend to occur in rivers and inland waters (Peñáz and Tesch, 1970 in Tesch, 1973; Hussein, 1983). However, other authors have shown that the different migratory habits of the sexes and biased sampling gear can alter sex ratios (Sinha and Jones 1966). Male eels migrate seawards sooner than female eels, for example, in the River Severn the mean age at maturity of the two sexes was 11.9 and 17.8 years respectively (Aprahamian, 1988). Males may show slower growth rates (Sinha and Jones, 1966) although leaving the system at a smaller size does not necessarily infer slower growth rates (Cripps, 1983). In a culture situation, although not directly comparable, male eels were found to have faster growth rates than females (Kuhlmann, 1974). Gonadal differentiation is held back somehow in eels, and its initiation is probably related to length rather than age (Beeckmann and Ollevier, 1987). The mechanism of sex determination of eels is not clear and appears to be influenced by genetic and environmental elements. It is reported by some authors as being labile, and under the influence of a range of environmental factors, such as population density (D'Ancona, 1950; Kuhlmann, 1974), and food quality and quantity (Kuhlmann, 1974). There is no evidence that salinity or temperature affect sex determination.

Growth of individual eels at the same age, and the incremental growth of the different age classes, were highly variable. Growth rates, expressed as absolute growth increments, did not reduce with increasing age in the populations studied, as one might expect (von Bertalanffy, 1957). It may be that the eels emigrate from the population, as silver eels, before the costs of maturation become heavy (Dekker, 1987). It is possible that the samples were not representative of the whole population, despite the relatively unselective nature of the electrofishing sampling method (Boccardy and Copper, 1963). It has been noted that large fish tend to move away from an electric field (Mann, 1976) and smaller fish, less than 2.5 cm are caught less readily (Mann and Orr, 1969). There may also be habitat segregation of different sized fish (Moriarty, 1972a; Sloane,1984).

Variation in growth is particularly high in *Anguilla anguilla* (Deelder, 1957) and was evident in the River Almond population. Growth variation was highest in age classes up to 2+, which were rapidly growing, contrary to increased variation when growth rates were low and nutritional conditions poor (Nikolsky, 1963). Variation of

a population can be described as the sum of environmental and genetic elements, (Purdom, 1974). Genetic variation is thought to be high in the eel (Kuhlmann, 1974). *Anguilla anguilla* is unique among teleosts in the peculiarities of its biological cycle and the high level of variation, even when compared with related species, for example, the conger eel *Conger conger*, which has a similar biological cycle except that it is wholely marine and more localised (Rodinó and Comparini, 1978). According to the theory of Schmidt (1922), the spawning grounds of *Anguilla anguilla* are confined to the Sargasso Sea, to which eels migrate up to 4,000 miles. The high degree of variability is thought to be a result of the large population contributing to a single genepool, where the degree of polymorphism is related to population size (Kimura and Ohta, 1971). Other theories which may explain the maintenance of variation in the genepool rely upon the wide distribution of *Anguilla anguilla,* that is, North-South from Iceland to the Canary Islands and East-West from the Black Sea to the Bermuda Islands (Tesch, 1973). These include 'niche variation hypothesis' where high genetic variation is an adaptive strategy in a temporally and spatially heterogeneous habitat (Somero and Soulé, 1974) and the differential survival of genotypes under different environmental conditions (Koehn, 1970).

There is probably a selective advantage in possessing a high degree of variation in the population, and of growth rate in particular, where environmental conditions are variable and often sub-optimal. There is no known mechanism which allows the return of offspring to the river system in which their parents matured, and hence no self-maintaining populations or means of differences accumulating over a number of generations (Williams *et al.*, 1973). *Anguilla anguilla* is an indigenous warm water species (Sinha and Jones, 1967a; Kuhlmann, 1974) and is near the extreme of its geographical range in Scotland, where it shows greater variation and slower growth than the same species from the Mediterranean, (Kuhlmann, 1974). All 17 species of *Anguilla* breed in sub-tropical or tropical seas and 12 are confined to such areas, whereas 5 undergo, often extensive, migration from more temperate waters (Bruun, 1963).

Thus, it becomes evident that *Anguilla anguilla* is able to respond to a wide range, of often unfavourable conditions, through a plastic growth rate which manifests itself in extreme differential growth, although it seems likely that the risks of mortality for slow growing individuals will be increased (Alexander, 1982).

3.5 SUMMARY

1. The reproduceability of the age determination methods was good and the age recorded by the two methods agreed in 79 % of the otoliths examined. There was a strong correlation between otolith length and eel length, $r = 0.95$; $p<0.0001$

2. There were a greater number of smaller and younger eels at Site 1, although biomass was similar at the two sites.

3. The weir between the two sites created a partial barrier to upriver migration which fore-shortened the effect that migration distance has on population structure.

4. Growth was highly variable and there was extreme overlap between length and weight of eels in adjacent age classes. Growth variation was highest in younger age classes which were growing rapidly.

5. Incremental growth of the population was similar to that found in other temperate waters.

6. Growth rate may have been reduced by population density at Site 1 and was higher in sexually differentiated eels. Growth rate was highest in the youngest age classes although there was no consistent decrease with increasing age.

CHAPTER 4

OTHER FACTORS AFFECTING GROWTH

4.1 INTRODUCTION

This chapter refers to other, more extrinsic factors affecting growth of the whole eel population, or individual eels. These include parasitism, disease, abnormalities, competition, predation, human intervention and environmental factors such as water temperature, food supply and daylength.

4.2 PARASITISM

Exotic species of nematode, that is, *Anguillicola crassa* (Kuwahara *et al.*, 1974) and *Anguillicola australiensis* causing serious infections of the swimbladder in *Anguilla anguilla*, have recently been reported (Egusa, 1979; Sarti *et al.*, 1985).

The presence of *Anguillicola crassa* in England was confirmed in 1987 (Kennedy, pers.comm.) and whether it was present in eels in Scotland was investigated in the present study. Eels were examined for skin ectoparasites and parasites of the gut cavity, swimbladder and organs associated with the gut. No attempt was made to examine other internal organs and hence the parasites recorded do not represent the complete parasitic fauna of eels in the River Almond.

4.2.1 Methods: (The general sampling procedure and sampling methods are described in Chapter 1.2.1). Parasites of 284 eels and 114 glass eels, sampled at monthly intervals over a 13 month period from Sites 1 and 2 on the River Almond, were investigated and their microhabitat preferences noted. Parasites were examined alive where possible, but frozen fish were also examined, as it has been observed that freezing does not cause loss of parasites from eels (Kennedy and Lord, 1982).

The following fixative and preservative methods were used: Protozoa were examined as a smear on a microscope slide, with a light microscope at magnification x 40 and preserved in 10 % formalin; Cestoda and Acanthocephala were relaxed in distilled water in a refrigerator, fixed in hot 70 % formalin and preserved in formol-acetic acid (FAA= 90 % absolute alcohol + 5 % formaldehyde (40 %) + 5% glacial

acetic acid) and Nematoda were fixed in hot 70 % alcohol and preserved in FAA .

The variations in the occurrence and intensity of infestation of the parasites observed were analysed in relation to sampling period, host habitat and the characteristics of the eel population. The significance of differences between the samples with respect to site and maturity of the host were tested by means of Mann-Whitney *U* -tests and the effects of host length and weight by means of Spearman rank correlation coefficients (Siegel, 1956).

4.2.2 Results: *Anguillicola crassa* was not present in the eel samples examined. However, two protozoan species, *Ichthyophthirius multifiliis* (Forquet, 1876) and *Octomitus truttae* (Schmidt, 1920) (=*Hexamita truttae*) and three metazoan species, *Bothriocephalus claviceps* (Goeze, 1782), *Acanthocephalus anguillae* (Müller, 1780) and *Raphidascaris acus* (Bloch, 1779) were recorded. One species, *Acanthocephalus anguillae* had not previously been reported from Scotland. (Identification of the metazoan parasites was verified by C. Kennedy).

The prevalence of infestation, that is, the percentage of the population infested and the intensity of infestation, that is, the number of parasites per infested fish, of parasite species in elvers and eels at both sites are shown in Table 4.1

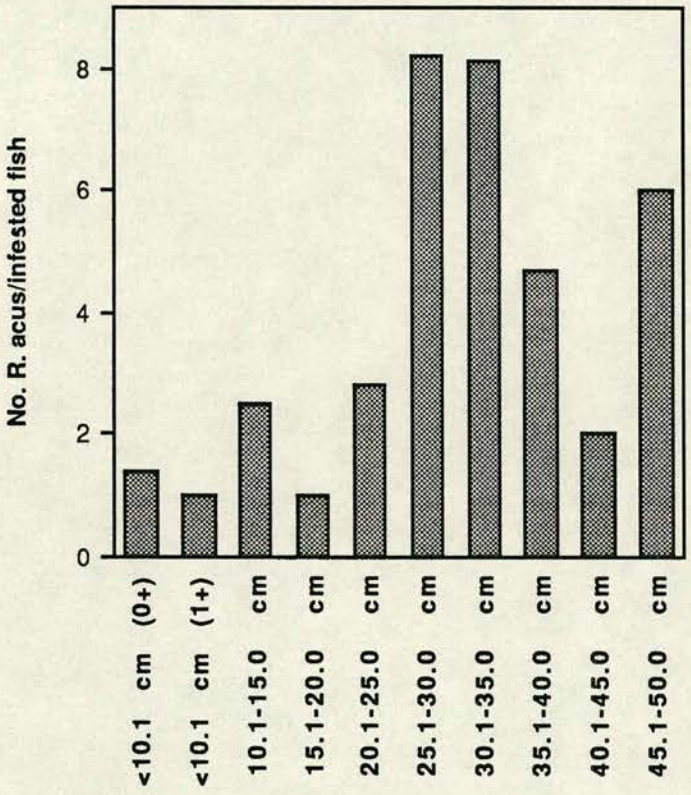
TABLE 4.1 Prevalence and intensity of infestation of parasite species in elvers and eels at Site 1 and Site 2

Parasite	Site	Prevalence		Intensity		Range	
		elvers	eels	elvers	eels	elvers	eels
Ichthyophthirius multifiliis	1	-	0.028	-	-	-	-
	2	-	-	-	-	-	-
Octomitus truttae	1	0.013	-	-	-	-	-
	2	0.088	-	-	-	-	-
Bothriocephalus claviceps	1	0.013	0.019	3.0	1.8	3	1-4
	2	-	-	-	-	-	-
Acanthocephalus anguillae	1	0.013	0.005	1.0	1.0	1	1
	2	-	0.029	-	1.0	-	1
Raphidascaris acus	1	7.500	21.500	1.0	4.0	1	1-20
	2	5.900	27.100	1.0	6.5	1	1-40

The only ectoparasite recorded was *Ichthyophthirius multifiliis* which was found in the epidermis of eels over 15 cm length at Site 1, between December and April. Another protozoan *Octomitus truttae* was found in the intestine of elvers at both sites in August. Both protozoan species and the cestode *Bothriocephalus claviceps* are typical eel parasites. *Bothriocephalus claviceps* was present at Site 1, where it was situated in the anterior part of the intestine of eels up to 25 cm length, between September and November. The acanthocephalan *Acanthocephalus anguillae* was present at both sites, in the intestine of eels up to 40 cm length, from April to September. The most common parasite was the nematode *Raphidascaris acus* which was present at both sites. It occurred throughout the year and across the size range of eels, where it occupied various microhabitats, including intestine, stomach, liver and body cavity. A non-specific fish leech *Hemiclepsis marginata* (Müller, 1974) was present in the benthic samples (Table 2.5) and the gut contents (Table 2.7) but was not observed on eels, it is described as being commonly found resting free (Fitter and Manuel, 1986).

Of the eels sampled 39.1 % were parasitized, although this was probably an underestimate because the total parasite fauna was not examined. Any differences in the parasitic fauna of male and female eels, associated with ecological differences between the sexes (Dogiel *et al.*, 1958) was not apparent in the small number of sexually differentiated eels examined. Infestation with *Raphidascaris acus* did not vary significantly between Site 1 and Site 2; $p<0.414$, and the data from both sites were combined. Population samples consisted of 82.4 % undifferentiated eels, 16.9 % females and 0.7 % males. Infestation of female eels with *Raphidascaris acus* was significantly higher (median=4) than immature eels(median=1); $p<0.05$, although this may also reflect increasing intensity of infestation with size, as females are larger than immatures of the same age. There were significant correlations between intensity of infestation with *Raphidascaris acus* and eel length and weight; $p<0.01$. The effect of eel size on the intensity of infestation with *Raphidascaris acus* is shown in Figure 4.1.

FIGURE 4.1 Effect of size of eel on intensity of infestation with *Raphidascaris acus*



Parasitic infestation, except for *Ichthyophthirius multifiliis*, showed a summer peak which is thought to be be related to the seasonal feeding activity of the eel and maturation of the parasite, rather than physiological synchronization of parasite and host life cycles (Conneely and McCarthy, 1986). A seasonality of infestation of *Raphidascaris acus* is shown in Figures 4.2 and 4.3 where prevalence and intensity of infestation are shown respectively. Infestation was highest in June and July, although the small number of eels caught in December and March were parasitised, and resulted in high values for prevalence of infestation in these months, which is misleading.

FIGURE 4.2 Prevalence of infestation of *Raphidascaris acus*

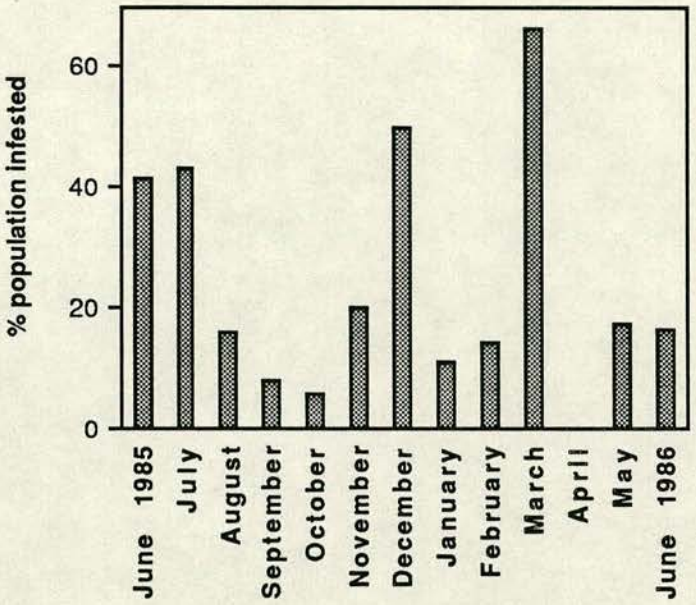
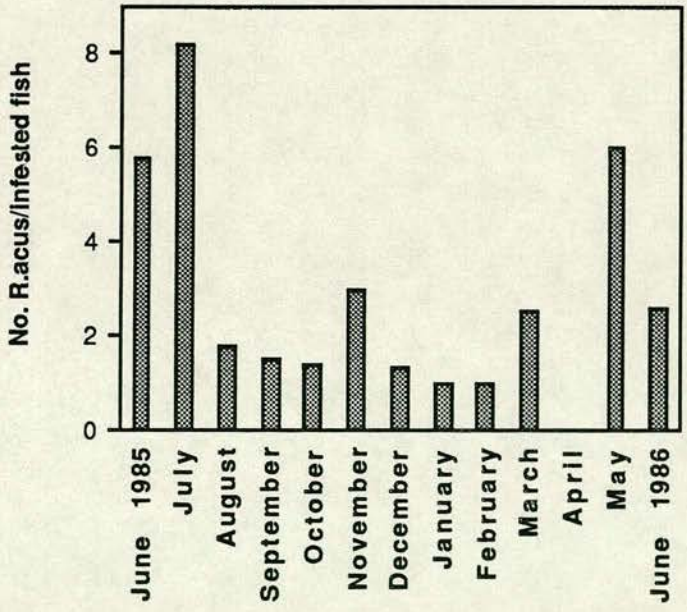


FIGURE 4.3 Intensity of infestation of *Raphidascaris acus*



4.2.3 Discussion: The presence of a parasite fauna containing species with indirect lifecycles, where particular species of invertebrates and fish are required for completion of the life cycle, are a useful indicator of the previous and likely future ecology of the host. This is particularly so with a migratory species like the eel with marine, coastal and freshwater periods of the life cycle. All parasites identified were typical of freshwater. The three metazoan parasites had crustacean intermediate hosts, that is, *Bothriocephalus claviceps* and *Raphidascaris acus*, a copepod, and *Acanthocephalus anguillae*, an isopod, which could be transmitted directly or via a fish secondary intermediate host or final host.

In the River Almond *Raphidascaris acus* occurred in all size groups of eels, contrary to observations where they were found to be restricted to larger fish (Conneely and McCarthy, 1986). This was explained as a requirement for a fish intermediate host (Moravec, 1971, in Conneely and McCarthy, 1986) where there was no evidence of copepods in stomach contents (Moriarty, 1972b). However, copepods were found in the stomach contents of elvers (Table 2.7) and in the benthic samples (Table 2.5) in the River Almond. This suggests that infestation of elvers and the largely non-piscivorous population of eels in the River Almond occurred by ingestion of infected copepods. This would also explain the occurrence of *Bothriocephalus claviceps* in elvers, another parasite which normally involves a fish intermediate host (Ginetsinskaya, 1958). There is experimental evidence that eels can become infected by ingestion of copepods infected with procercoids (Jarecka, 1964, in Conneely and McCarthy, 1986).

Apart from *Anguillicola* spp., and possibly some Acanthocephala, metazoan parasites seldom reach pathogenic proportions in *Anguilla anguilla* (Tesch, 1973). They may hinder the growth of individual fish, where the rate of food consumption is related to the condition of the fish itself (Nikolsky, 1963), but their importance in affecting the growth of an entire population may be considered as minor (Van Oosten, 1944). There were no significant differences between length and weight of parasitized and non-parasitized immature eels of the same size; $p>0.05$.

The protozoan parasites, however, can reach pathogenic proportions. *Ichthyophthirius multifiliis* is an important fish pathogen (Sommerville, pers.comm.). It is not specific to eels but is often found in them, which may be a result of their crowding behaviour, often in benthic areas of reduced water exchange (Tesch, 1973). *Octomitus truttae* is a typical debility parasite, which is indicative of other stress factors (Uzmann *et al.*, 1965).

4.3 DISEASE

Bacterial and viral infections cause the greatest proportion of disease in eels (Tesch, 1973). 'Cauliflower disease', caused by a viral pathogen, has been reported from the River Tweed, Scotland (Hussein and Mills, 1982) and was observed in three eels during this study in the River Almond. These eels had tumour-like swellings on both upper and lower jaws, which may have affected food intake as they all had empty stomachs. Any effect on growth could not be assessed because of the small sample size

4.4 ABNORMALITIES

Certain abnormalities were noted in the eel samples which could have been as a result of injury, as described later, or an hereditary anomaly. There were eels whose normally superior lower jaws were either, extremely elongated, inferior or equal in length to the upper jaw.

4.5 COMPETITION

Competition for food may occur during April to September when the eel is actively feeding. This may be lessened by the increase in abundance of certain benthic organisms (Sinha and Jones, 1967b) although many aquatic invertebrates may have emerged and flown, or died, by the summer (Varley, 1967). Intraspecific competition was thought to be a factor influencing the growth of smaller eels in a Welsh river (Sinha and Jones, 1967a) and at Site 1 in the River Almond (Chapter 3). The balance of competition between one species and another changes as the abundance of the food organism changes (Hartley, 1948) but the eel under natural conditions and with respect to native species appears to have found its niche (Tesch, 1985). The fish species present, and the numbers caught per m^2, during electrofishing in the River Almond, are shown in Table 4.2. Atlantic salmon *Salmo salar* are found in the River Almond (Campbell, 1984) but were not present in the samples.

TABLE 4.2 Fish species in the River Almond

Fish	Number. m^{-2}	
	Site 1	Site 2
Eels	0.14	0.05
Elvers	6.72	0.19
Neomacheilus barbatulus	0.005	0.06
Gasterosteus aculeatus	0.02	-
Phoxinus phoxinus	0.04	0.02
Platichthys flesus	0.003	-
Salmo trutta	-	0.002
Cottus gobio	-	0.001

4.6 PREDATORS

There was evidence of predation by eels (Chapter 2), grey heron *Ardea cinera* and humans. There were sightings of gulls *Larus* spp. and mink *Mustela* sp. which are capable of taking eels. Of the other fish species present, the salmonids are possible predators of small eels, although no stomach contents were examined.

4.7 HUMAN INTERVENTION

Obstructions to migration

The weir, situated between Site 1 and Site 2 on the River Almond is three metres high, with a fish ladder built into the west side. There was evidence to suggest that the weir acts as a partial barrier to migration of both glass eels, where migration from Site 1 to Site 2 was delayed by 2 to 3 months (Chapter 6), and small yellow eels (years 3+ and 4+), where densities were significantly higher at Site 1 (Chapter 3). This is not unusual in eels which, unlike anadromous species such as salmon and lampreys *Lampetra fluviatilis* and *Petromyzon marinus* are less intent on continuing their migration (Tesch, 1966 in Tesch, 1973).

Angling

There is no commercial fishery for glass eels, yellow eels or silver eels, however, eels are taken by anglers and are often treated roughly if they are put back. There was one eel in the samples with a mis-shapen jaw which could have been caused by a hook.

Mechanical

Other injuries can be caused by turbines or boat propellors. There were eels in the samples whose pectoral fins were either, fore-shortened, mere stumps or missing altogether.

Pollution

Pollution, of a mostly organic nature, can reach high levels in the River Almond (Chapter 2). The eel is particularly tolerant, but growth might be affected, although permanent damage seldom occurs (Tesch, 1973). Heavy metals and other pollutants, such as polychlorinated biphenols, may affect reproductive capacity (Westernhagen *et al.*, 1981).

4.8 ENVIRONMENTAL FACTORS

Water temperature was probably the limiting factor in the feeding activity and hence growth of eels in the River Almond. There was little or no growth during the winter, as seen in the otolith check, and little or no feeding below 10 °C (Figure 2.7). Similar effects of low temperature are seen in other species of eel, for example, the Japanese eel *Anguilla japonica* whose growth rate is reduced below 14 °C and feeding ceases at 10 °C (Matsui, 1952). This is thought to be a physiological reaction to temperature extreme, as a similar effect is seen at high temperatures, for example, 31°C in an Egyptian lake (Ezzat and El-Seraffy, 1977). Examination of continuous temperature recording data indicated that there were less than 150 days from June 1985 to May 1986 when water temperatures were above 10 °C in the River Almond (Chapter 2.2.1).

Paucity of food has been reported as the major growth limiting factor of eels in Lake Windermere, England (Frost, 1945) and in warmer climes, where there are no lower temperature limitations (Bertin, 1956; D'Ancona, 1960). In the River Almond food was present throughout the year, although the type of food may not have been optimal for growth, that is, mainly naid worms and chironomid larvae.

Daylengths are long during the summer in Scotland and may limit the growth of eels whose feeding activity is mainly nocturnal. For example, using equations 1.22, 1.31 and 1.55 (Iqbal, 1983) the number of hours of darkness during the 150 days when water temperatures were above 10 °C, was 1650 h at 56 °N (Edinburgh) compared with 1946 h at 36 °N (Gibraltar) during the same period. Therefore, in one year temperature could restrict feeding to 21 weeks of which light may reduce this to 19 weeks. Thus, it would appear that environmental conditions in Scotland are not optimal for the growth of eels.

4.9 SUMMARY

1. The swimbladder nematode *Anguillicola crassa* was not observed in eels from the River Almond.

2. Five species of parasite were recorded, the most common of which was a nematode *Raphidascaris acus*. One species, *Acanthocephalus anguillae* had not previously been reported from Scotland.

3. There was evidence of infestation with *Raphidascaris acus* and *Bothriocephalus claviceps* by ingestion of infected copepods.

4. There was a summer peak of parasitic infestation which was probably related to feeding activity of the host.

5. There was a significant increase in intensity of infestation with increasing weight and length of host.

6. There was no evidence that growth was affected by parasitic infestation.

7. 'Cauliflower disease' was observed in three eels in the River Almond.

8. Water temperature and daylength during June 1985 to June 1986 were suited to eel feeding activity and growth for approximately 19 weeks at 56 °N.

CHAPTER 5

GROWTH AND BODY COMPOSITION

5.1 INTRODUCTION

Changes in the relative proportions of the four major components of eel tissue, that is, water, protein, lipid and ash have been shown to be related to body size in wild eels (Gallagher *et al.*, 1984a) and in cultured eels (Degani *et al.*, 1986). Dry weight, wet weight and length have been used as indices of size. Length has also been used as an index of age of wild eels, where no independent estimate of age was determined (Gallagher *et al.*, 1984a).

In this chapter the effect of age, or growth rate, on body composition of eels in the River Almond is assessed independently of size. This is achieved by determining age through interpretation of growth rings in the otoliths. Dry weight was used as an index of size and length was used as an index of condition. The influence of sexual maturation on body composition was assessed by separate analysis of immature and maturing fish, and the effect of seasonality on body composition was examined.

5.2 METHODS (The general sampling procedure and sampling methods are described in Chapter 1.2.1)

Proximate body composition analyses were performed on 252 eels and pigmented elvers sampled from the River Almond at monthly intervals from June 1985 to June 1986. Length was measured to the nearest 0.1 cm and weight, minus the gut contents, was recorded to the nearest 0.01 g. The food organisms in the stomach contents (Chapter 2), age (Chapter 3) and sex (Chapter 1) of individual fish had been previously recorded. Each fish was assigned a number and tagged with a card tag bearing pencil inscriptions, which were immune to the solvent used in later extraction.

Whole frozen eels were dried to constant weight in a model EF2 Edwards Freeze Drier and then stored in a dessicator at room temperature. The samples were weighed to 0.001 g on a Sauter RE164 balance and were wrapped in paper towel envelopes. Crude lipid was extracted by refluxing in chloroform, in a Soxhlet apparatus for 18 hours. This was found to give a constant lean weight. The samples were dried at 105 °C for 12 hours and stored in a dessicator and the lean weight determined to 0.001 g. Lipid weight was determined to 0.001g by difference of lean

weight from dry weight. Ash was determined by measurement of the residue after heating in a muffle furnace at 550 °C for 12 hours. Protein was determined by subtraction of ash from the lean weight, where the very low carbohydrate fraction was considered as negligible. Fish contain low levels of glycogen and other carbohydrates, for example, 0.1 % wet weight of perch *Perca fluviatilis* (Craig, 1977), although the levels quoted are variable up to 4 % wet weight (Smith, 1982). Water content was expressed as a percentage of the lean weight, in order to remove the effect of the negative correlation of percent water with lipid, which is found in fish (Love, 1980). Water content was calculated using the equation: water = 100 (wet weight (g) - dry weight (g) /wet weight (g) - lipid (g)). Protein, lipid and ash were expressed as a percentage of the dry weight.

Samples from two sites (Chapter 1) were pooled to increase sample sizes. It was felt that any effect of site would be masked by variation due to size, age and maturity. The effect of site could not be tested statistically, once the effect of these other variables had been removed because the sample sizes were too small. A similar situation was found when the effect of seasonality was tested, the sample sizes were too small to exclude the effect of variation in the size of eels caught in different seasons.

Eels excluded from statistical analysis were: those under 1 g, because of the high error involved in the measurement of minute fractions of the body constituents; one eel because of an error during analysis; two male eels and the eels whose age could not be determined. Of the 211 fish that remained, 173 immatures and 38 maturing females were separated for statistical analysis.

Change in body composition was evaluated by multiple regression of the measured constituents (y) with dry weight (x_1), age (x_2) and length (x_3). The models tested were:- $y = a+bx_1+bx_2+bx_3$; $y = a+bx_1+bx_2$ and $y = a+bx_1+bx_3$. The eel population was not normally distributed with respect to age and size. Proportional data are not normally distributed and arcsine transformation of the body composition parameters was required to meet the conditions for statistical inference in the regression analysis (Sokal and Rohlf, 1969). The purpose of multiple regression was to estimate and fit a structural model to explain variation in the observations of y in terms of the independent variables x_1, x_2, and x_3. Partial regression coefficients were tested for significance using a *t* -test, and estimation of the relative magnitudes of the contributions of the independent variables was made using the standardized form of the regression equation. Standardization transforms the conventional partial regression coefficients (b) from their original measurement units to standard deviates

(b'), also known as beta coefficients, thus giving the rate of change in standard deviation units of y per one standard deviation unit of x_n, where all other x variables are kept constant.

The best fit for the observed data was obtained using dry weight together with length or age and the explained sum of squares did not increase appreciably with three x variables. The independent variables were correlated with each other, which explains differences in signs and magnitudes of the coefficients when different variables are regressed. With dry weight held constant, a significant effect of age was related to growth rate and a significant effect of length was related to condition, where condition is an indication of the relationship between weight and length.

It should be noted that in instances where the body composition constituent is included in the numerator and the denominator of the equation, the slope of the graph does not truly represent the relationship between the variables. The lack of independence in proportional data does present a problem with interpretation due to possible autocorrelation and masking effects. Standardization of such data, is discussed by Le Cren (1951) for the calculation of body condition in the perch and developed by Barbour (1984) for analysis of body composition in Arctic charr *Salvelinus alpinus*. This was not attempted for lipid or protein of *Anguilla anguilla* in this study because standardization involves the use of empirical constants derived from the weight to length relationship, which is not suitable for *Anguilla anguilla* (Sparre, 1979). In the studies cited, concerning body composition of eels, the constituents have not been standardized and it was considered valid to do likewise here. It should be noted that in these studies water is calculated as a percentage of wet weight and not lean weight as presented here.

Water as a percentage of the lean weight is reduced to a virtual constant in relation to size in contrast to the percentage of the wet weight which decreases with increasing size (Gallagher *et al.*, 1984a; Degani *et al.*, 1986) Thus, water can be dismissed as a variable and the relationship of protein, lipid and ash can be investigated.

5.3 RESULTS

The weight of the eels analysed varied from 1.18 g to 183.41 g and length ranged from 10.0 cm to 46.3 cm although most were small individuals (medians = 11.74 g and 21.7 cm), which were sexually undifferentiated (82.0 %) and the remainder were maturing females. The range of body composition was particularly wide in immature fish (Table 5.1 and Figures 5.1 and 5.2).

FIGURE 5.1 Relationship between water (% lean weight), protein, lipid, and ash (% dry weight) and dry weight (g) of immature eels

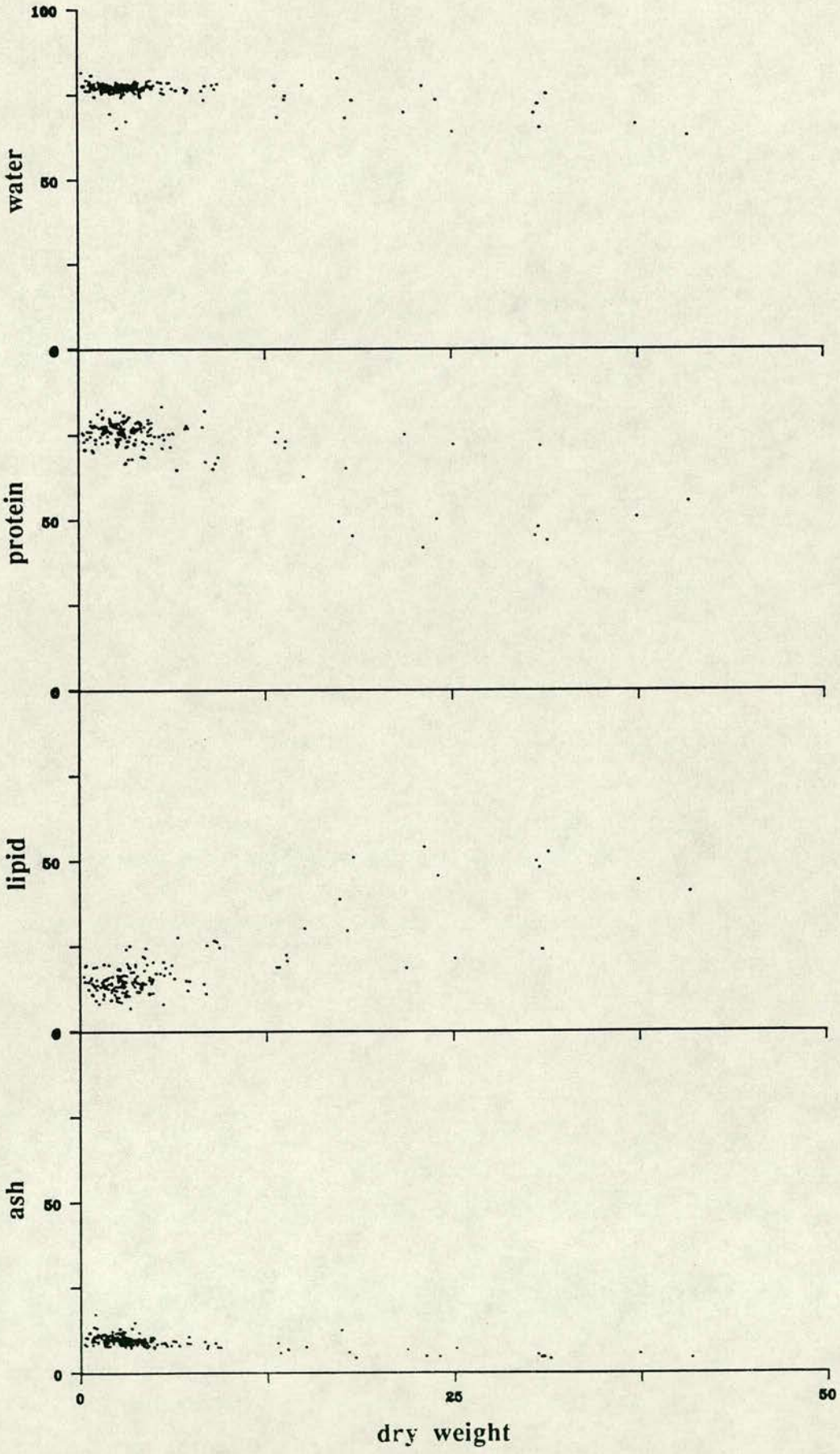


FIGURE 5.2 Relationship between water (% lean weight), protein, lipid, and ash (% dry weight) and dry weight (g) of female eels

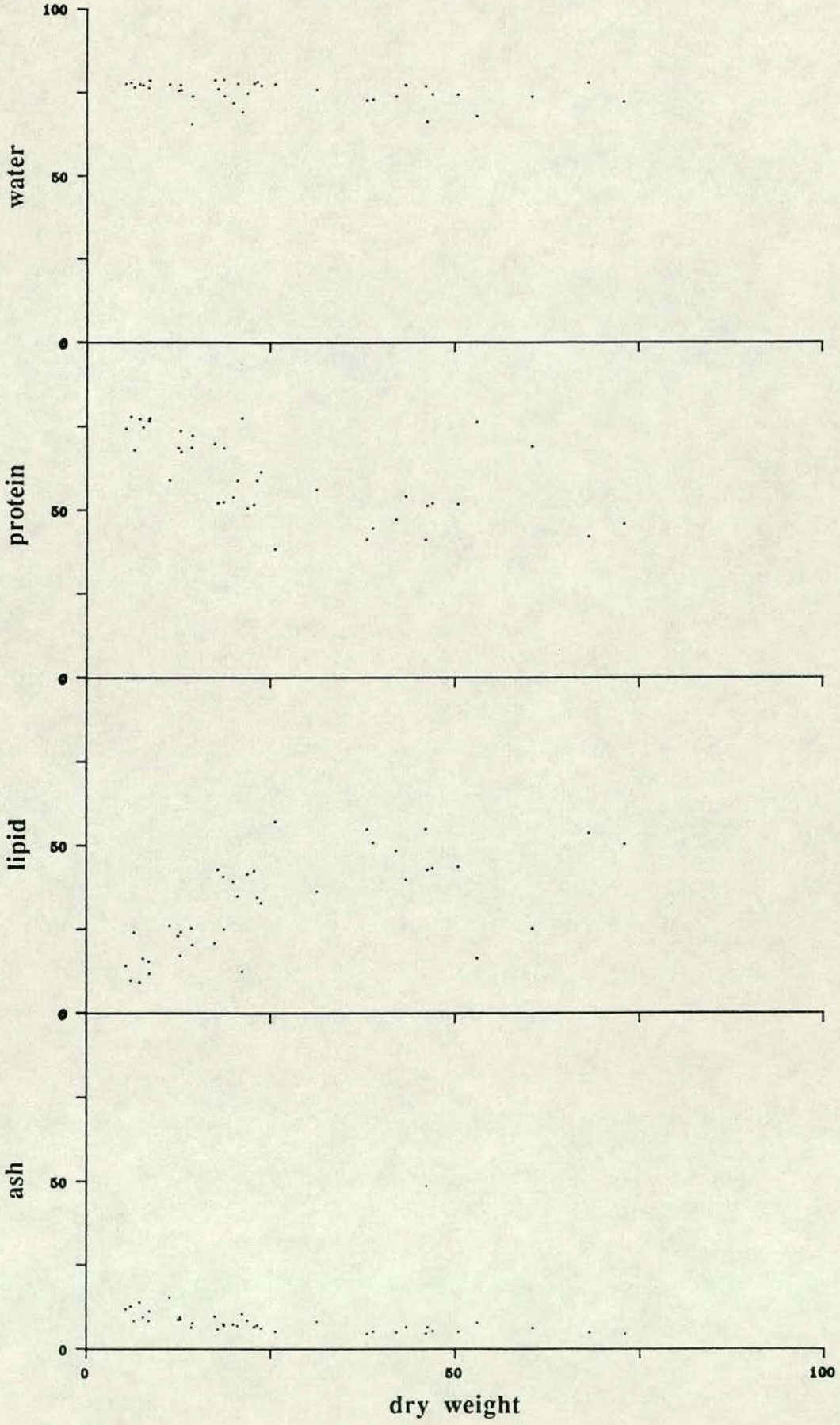


TABLE 5.1 Body composition (% dry weight) of immature and female eels in the River Almond (l=%lean weight)

	Lower Quartile	Median	Upper Quartile	Mini-mum	Maxi-mum
IMMATURE					
weight (g)	7.60	11.70	18.79	1.18	81.02
length (cm)	19.1	21.7	24.4	10.0	37.5
water (l)	75.96	76.83	77.59	62.32	81.53
protein	72.74	75.76	77.48	41.39	83.29
lipid	12.71	14.60	18.62	6.72	53.94
ash	8.32	9.29	10.15	3.99	17.19
FEMALE					
weight (g)	40.91	61.96	97.82	21.09	183.41
length (cm)	32.4	34.8	39.3	25.3	46.3
water (l)	73.07	75.85	77.11	65.03	78.31
protein	51.24	58.75	72.57	38.01	77.83
lipid	19.42	33.58	42.75	8.99	56.98
ash	5.53	7.05	8.79	4.23	15.28

5.3.1 Body composition and dry weight

Protein and ash levels decreased whilst lipid increased with increasing dry weight in immature and female eels (Figures 5.1 and 5.2). The regression coefficients for the relationship between the body composition constituents and dry weight were significant, $p<0.0001$ in immature and female eels (Tables 5.2 and 5.3). There was correlation between all the x variables.

5.3.2 Body composition and length

Protein level significantly increased; $p<0.001$, lipid level decreased significantly; $p<0.05$ and ash was not affected with increasing length of immature eels. The body composition of female eels was not significantly affected by length (Table 5.2).

5.3.3 Body composition and age

Protein levels significantly increased and lipid levels significantly decreased, $p<0.05$, with increasing age of immature eels (Table 5.3). Age did not significantly affect the body composition of female eels. Age contributed to more variability than length in both immature and female eels as shown by the higher beta coefficients for protein and lipid.

5.3.4 Body composition and season

The data were grouped into seasons: June, July and August (summer); September, October and November (autumn); December, January and February (winter) and March, April and May (spring). The body composition constituents were regressed simply against dry weight for each season in immature eels and in the summer for female eels but sample sizes of female eels were too small in the other seasons. All regression coefficients were significant, $p<0.05$ (Table 5.4).

TABLE 5.2 Multiple regression of body composition constituents (% dry weight) with dry weight (x_1) and length (x_3): $y = a+bx_1+bx_3$

(l=%lean weight, se=standard error, df=degrees of freedom, *** = p<0.0001, ** = p<0.001, * = p<0.05)

	se		b′		significance of b	
	x_1	x_3	$b'x_1$	$b'x_3$	$b\ x_1$	$b\ x_3$
IMMATURE (df=170)						
water(l)	0.0006	0.0008	- 0.0000	+ 0.0000	***	
protein	0.0013	0.0017	- 0.0032	+ 0.0013	***	*
lipid	0.0010	0.0013	+ 0.0032	- 0.0013	***	**
ash	0.0003	0.0004	- 0.0005	+ 0.0001	***	
FEMALE (df=35)						
water(l)	0.0011	0.0037	- 0.0001	- 0.0002		
protein	0.0038	0.0141	- 0.0007	+ 0.0001		
lipid	0.0027	0.0095	+ 0.0008	- 0.0007	***	
ash	0.0005	0.0016	- 0.0001	- 0.0002		

TABLE 5.3 Multiple regression of body composition constituents (% dry weight) with dry weight (x_1) and age (x_2): $y = a+bx_1+bx_2$

(l=%lean weight, se=standard error, df=degrees of freedom, *** = p<0.0001, * = p<0.05)

	se		b′		significance of b	
	x_1	x_2	$b'x_1$	$b'x_2$	b x_1	b x_2
IMMATURE (df=170)						
water(l)	0.0005	0.0020	- 0.0001	+ 0.0011	***	
protein	0.0009	0.0041	- 0.0022	+ 0.0071	***	*
lipid	0.0008	0.0033	+ 0.0021	- 0.0061	***	*
ash	0.0002	0.0009	- 0.0003	- 0.0001	***	
FEMALE (df=35)						
water(l)	0.0005	0.0064	- 0.0001	- 0.0046		
protein	0.0017	0.0207	- 0.0004	+ 0.0088	***	
lipid	0.0013	0.0165	+ 0.0038	- 0.0088	***	
ash	0.0002	0.0027	- 0.0001	+ 0.0012	***	

TABLE 5.4 Regression of body composition parameters (y) (% dry weight) with dry weight (x) : $y = a + bx$ (w=%wet weight, se=standard error, df=degrees of freedom, s=significance of b, *** = p<0.0001, ** =P<0.001, * = p<0.05)

	summer (df=76)		autumn (df=35)		winter (df=21)		spring (df=33)	
	se	s	se	s	se	s	se	s
IMMATURE								
moisture (w)	0.0004	***	0.0006	***	0.0012	***	0.0015	***
protein	0.0011	***	0.0012	***	0.0021	***	0.0013	*
lipid	0.0008	***	0.0010	***	0.0017	***	0.0010	***
ash	0.0002	***	0.0004	***	0.0004	***	0.0004	***
FEMALE	(df=26)							
moisture (w)	0.0001	***						
protein	0.0014	*						
lipid	0.0013	**						
ash	0.0002	***						

5.4 DISCUSSION

Body composition was extremely variable in the River Almond eels (Table 5.1) as has been found in other studies of wild eels. For example, *Anguilla rostrata* of weight range 3.95 to 197.26 g showed the following variation in body composition: water 64.68 - 80.70 % (wet weight); protein 42.90 - 65.76 %; lipid 15.70 - 64.03 % and ash 3.30 - 11.50 % (dry weight) (Gallagher *et al.*, 1984a).

Changes in body composition with relation to increasing size of eel, that is, increasing lipid levels and decreasing proportions of protein and ash were apparent in this study (Figure 5.1) This is consistent with other studies (Gallagher *et al.*, 1984a; Degani *et al.*, 1986) and increasing age in most animals (McDonald *et al.*, 1981).

Body composition is also influenced by factors such as quantitative differences in the diet and physiological state. Qualitative differences in the diet are unlikely to influence body composition unless they affect the proportions of the constituents that cannot be synthesized from food materials or reserves (Love, 1980). The diet, throughout the size range of eels in the River Almond, consisted mainly of oligochaete worms, chironomid larvae and other insects (Figure 2.11) and thus it is improbable that changes in body composition are related to qualitative differences in the diet. Quantitative changes in the diet occur seasonally in the River Almond, where feeding activity appears to be related to water temperatures above 10 °C (Chapter 2). It was likely that nutritional condition was highest in the autumn, at the end of the feeding season, and lowest in the spring, after five months fasting. There was no significant change in the body composition of eels in different seasons but differences may have been masked by the wide size variation of the eels caught in different seasons.

The range for each body composition constituent was less in female than immature eels, although the range was wider than reported for larger, mature eels. For example, eels >530 g, have quite a constant body composition, that is, 54% moisture, 31.3% lipid and 10.6% protein (wet weight) (Boëtius and Boëtius, 1980). Extremely high lipid levels occur in mature eels in preparation for extensive migrations to spawning grounds (Tesch, 1973). The largest maturing individual in this study (183.41 g) was less than half the weight of mature eels (>400 g) and ovaries were in the early stages of development, which may account for the difference compared with mature fish. The lack of a significant effect of age and length on the body composition of female eels suggests that a threshold has been reached at differentiation beyond which body composition is less influenced by age and length. Sexual differentiation is considered to be dependent upon length (Kuhlmann, 1974) and in the present study female fish were longer than immature fish of the same age

(Chapter 3).

Older fish had a lower proportion of lipid than rapidly growing fish of similar weight. Lipid is the main energy store of most aquatic animals and is stored under non-limiting conditions in the liver or muscle of fish, and mainly in the muscle of eels (Dave *et al.,* 1974). Total lipid is an indicator of nutritional condition (Love, 1980). The slow growth of smaller eels in the wild (Gallagher *et al.*, 1984a) and under-developed eels in cultured populations (Gallagher *et al.*, 1984b; Degani and Gallagher, 1985) has been related to higher oxygen consumption rates than would be expected for their size, and low feed conversion efficiency.

The results in this study indicate that body composition is affected by growth rate, which in turn may be influenced by the physiological state of the fish. As it seems likely that glass eels have the same genetic potential for growth (Wickins, 1987) physiological differences would appear to be induced by environmental factors such as competitive stress. The increase in the proportion of protein with increasing age may be due to an inverse relationship with lipid. Ash was not significantly affected by age whereas there was a significant decrease in ash with increasing dry weight. Ash is less likely to change with age in absolute amount once the skeleton has formed although the proportion of ash will vary with size and condition. Eels that were longer than others of the same weight had lower lipid levels which was a reflection of condition and not necessarily an effect of age, although length and age were correlated. It appears slow growing eels or eels in low condition may be less likely to reach the threshold of condition required for sexual differentiation before succumbing to predation (Alexander, 1982).

5.5 SUMMARY

1. Body composition changed with size of eel. The proportions of lipid increased and protein and ash decreased with increasing size. Water was found to be almost constant when calculated as percent fat-free wet weight.

2. Growth rate affected lipid level, which was higher in rapidly growing eels than slow growing individuals of the same weight.

3. Condition also affected lipid level, which was higher in eels that were shorter than others of the same weight.

4. Sexually differentiated individuals were rapidly growing and in good condition, where age or length had no significant effect on body composition.

5. Body composition was not significantly affected by seasonality.

CHAPTER 6

GLASS EEL MIGRATION AND DEVELOPMENT DURING THE FIRST YEAR IN FRESHWATER

6.1 INTRODUCTION

Eels do not breed successfully in captivity. Induced breeding of *Anguilla anguilla* was first attempted in 1937 (Fontaine and Tuzet, 1937) and has developed to a point where larvae of *Anguilla japonica* survive 5 days (Yamamoto and Yamauchi, 1974). Eel culturists are dependent upon a natural source of stock which becomes available as part of an annual migration of glass eels into coastal and inland waters. If growth in culture is to be optimised and any adverse effects of transfer kept to a minimum, an understanding of the migration and the development of the glass eels is essential.

The life cycle of the eel involves a growth phase in continental waters of 'yellow eels' which migrate as adult 'silver eels' in late summer and autumn to spawning grounds in the Sargasso Sea (26 °N 56 °W). The leaf-like leptocephalus larva is carried back in the Gulf Stream to the European continental shelf in autumn where it metamorphoses into the 'glass eel', a transparent replica of the adult (Schmidt, 1912). It is generally accepted that each migration represents a single year class (Moriarty *et al.*, 1987a) but the time taken to cross the Atlantic is variously estimated between 2 to 3 years (Schmidt, 1923) or less than 2 years (Boëtius and Harding, 1985b). To avoid confusion, age is recorded from the time the glass eels enter freshwater (Sinha and Jones, 1967a).

Glass eels have migrated into coastal areas of the North Sea by December, having travelled in the Gulf Stream up the west coast of Britain, entering the North Sea from north to south rather than through the English Channel (Tesch, 1973). They do not migrate into inland waters until the outgoing freshwater reaches 9 °C, when they become more active at this temperature (Tesch, 1973). There are physiological changes associated with this transfer from seawater to freshwater. During this period they become increasingly negatively phototactic, which accompanies the change from planktonic to benthic life, and rheotactic, where the response to tidal flow helps with the inland migration. This is reported to be synchronized with spring tides (Cantrelle, 1981) and darkness (Tesch, 1973) and further affected by river outflow (Elie, 1979 in Cantrelle, 1984). The glass eels become gradually more pigmented and decrease in

length until Stage VI A iii $_2$ (Strubberg, 1913) is reached (Tesch, 1973). The term 'elver' is applied loosely both to glass eels and the pigmented stages (Moriarty, 1987a). In this study, glass eels are referred to as 'elvers' once pigmentation is complete rather than at the onset of pigmentation (Tesch, 1973), to allow their differentiation during pigmentation. Food is taken in very small amounts by glass eels up to Stage VI A iii $_2$ and in increasing amounts with further development. The food of glass eels in the River Almond included *Tubifex* spp. and naid oligochaete worms, *Cyclops* spp., mayfly, beetle, black fly and chironomid larvae (Table 2.8).

In this chapter the migration and development of glass eels during their first year in freshwater in the River Almond is described.

6.2 METHODS (The general sampling procedure and sampling methods are described in Chapter 1.2.1)

Sampling of glass eels and elvers was incorporated in the 13 month sampling programme of the eel population at 2 sites on the River Almond (Chapter 1). A further sampling point was made at the weir (Site 3) between both sites, where glass eels collected and the upstream migration was delayed for 2 to 3 months. Additional nightly inspection of the river by torch light and weekly electrofishing was undertaken in an attempt to locate the start of the migration and once this was achieved monthly sampling was continued. Water temperature and the diurnal and lunar state of the tide were recorded. Monthly samples of glass eels and elvers were weighed to the nearest 0.001 g and length measured to the nearest 0.1 cm. An estimate of population density was made from the number caught per m^2, although numbers were likely to be highly variable, (Cantrelle, 1984). The stage of pigmentation, according to the description of Strubberg (1913) was recorded. Stomach contents were analysed and monthly samples, minus gut contents, were bulked for proximate analysis (Chapter 5.2). Otoliths were removed for age determination (Chapter 3).

6.3 RESULTS

There were two glass eel migrations during the 13 month period of this study (Table 6.1). Glass eels were first sighted on 5/5/85 and 2/6/86 when the water temperature was 11.0 °C and 15.0 °C in the respective years. The number of glass eels was highest in June 1986 when there were 67 per m^2 at Site 1, but densities dropped to 6.35 per m^2 by July. During the 1985 migration the highest density recorded was 4.25 per m^2 in July. The first sighting of glass eels in 1985 coincided

with spring tides and darkness, but fell between spring- and neap-tides in daylight in 1986.

Glass eels were not caught at Site 2 until July in both years but accumulated at the weir (Site 3), between Site 1 and Site 2, where they could be found in cracks in the wall of the brick fish ladder and shallow pools near it. Samples of glass eels taken from Site 3 in May, June and July were not significantly different from each other with respect to weight (Mann-Whitney *U* -Test; $p<0.001$) or significantly different from glass eels at Site 1 in May, but were significantly different from glass eels at Site 1 in June and July. Glass eels at Site 1 were significantly different from each other in May, June and July. Glass eels were normally distributed with respect to weight at Site 3 in May, June and July and at the beginning of the migration at Site 1 in May. The distribution became increasingly skewed in successive months at Site 1 (Figure 6.1). Coefficient of variation (CV%) (CV% = standard deviation / mean x 100) increased at Site 1 from 17.5 % to 34 %, but decreased at Site 3 from 22.9 % to 17.9 %, from May to July.

All but one individual, whose stomach contained one chironomid larva, of the 60 glass eels sampled at Site 3 had empty stomachs, and intestines that were green in colouration and gall bladders which were full and yellow, whereas glass eels at Sites 1 and 2 were feeding actively (Chapter 2). Development of pigmentation was slower in glass eels at Site 3, which were at Stages VI A iii and VI A iv, whereas some of those at Sites 1 and 2 were fully pigmented, that is, Stage VI B (Strubberg, 1913). All glass eels had become fully pigmented elvers by October. Glass eels and elvers that were exposed to sunlight appeared to increase the intensity of pigmentation rapidly, due to the reaction of the melanophores to increased light intensity (Nicol, 1963). The pigment cells also react rapidly to match a change in the shade of background in the wild and in culture tanks (Seymour, 1984) but the reaction is reported to become less rapid with increasing size (Tesch, 1973).

The body composition of glass eels changed with growth and development into elvers at Site 1 but remained constant at Site 3 (Table 6.2). Water levels were particularly variable, within the range of 84.8 % to 71.2 %. The other parameters were expressed on a dry weight basis. Lipid levels were low in glass eels at Site 3, that is, below 9 %, whereas glass eels at Site 1, at the same time, had higher lipid levels. Lipid level was highest in elver samples in September.

TABLE 6.1 Development of glass eels and elvers during their first year in freshwater (T=temperature, $N.m^{-2}$ =number per square metre, n=sample size and sd=standard deviation)

Date	Site	T °C	$N.m^{-2}$	n	Length (cm)	sd	Weight (mg)	sd	Pigmentation
5/5/85	1	11.0	2.75	-	-	-	-	-	VIAiii/iv
14/5/85	1	13.0	3.95	55	7.1	0.3	228	40	VIAiii/iv
15/5/85	3	16.0	-	110	7.1	0.4	231	53	VIAiii/iv
6/6/85	3	17.0	-	68	7.1	0.3	223	31	VIAiii/iv
23/6/85	1	16.0	1.30	79	7.2	0.3	291	79	VIAiii/iv/VIB
4/7/85	3	16.0	-	73	7.1	0.4	229	41	VIAiii/iv
7/7/85	1	16.0	4.25	85	7.3	0.5	332	116	VIAvi/VIB
7/7/85	2	16.5	0.10	2	6.5	0.1	205	31	VIAiv
8/8/85	1	13.0	2.45	20	7.4	0.4	339	124	VIAiv/VIB
8/8/85	2	13.0	1.25	20	7.5	0.6	387	155	VIAiv/VIB
11/9/85	1	12.5	1.75	20	7.4	1.0	312	138	VIAiv/VIB
11/9/85	2	12.5	0.70	14	7.9	0.8	526	73	VIAiv/VIB
10/10/85	1	10.0	1.05	21	7.2	1.8	475	367	VIB
10/10/85	2	10.0	0.30	6	7.0	0.5	330	76	VIB
21/11/85	1	5.0	0.05	1	6.6	-	223	-	VIB
5/1/86	1	1.5	0.20	4	7.1	0.4	317	45	VIB
26/1/86	1	2.0	0.05	1	6.0	-	209	-	VIB
27/2/86	1	1.0	0.20	4	8.2	1.2	574	352	VIB
27/2/86	2	1.0	0.10	2	8.2	0.9	549	184	VIB
24/4/86	1	8.0	0.05	1	7.1	-	308	-	VIB
2/6/86	1	15.0	67.00	20	7.0	0.4	254	50	VIAiii/iv
3/7/86	1	18.5	6.35	20	7.0	0.3	286	64	VIAiii/iv
3/7/86	2	18.5	0.05	1	8.2	-	500	-	VIAiv

FIGURE 6.1 Change in weight distribution of glass eels and elvers at Site 1

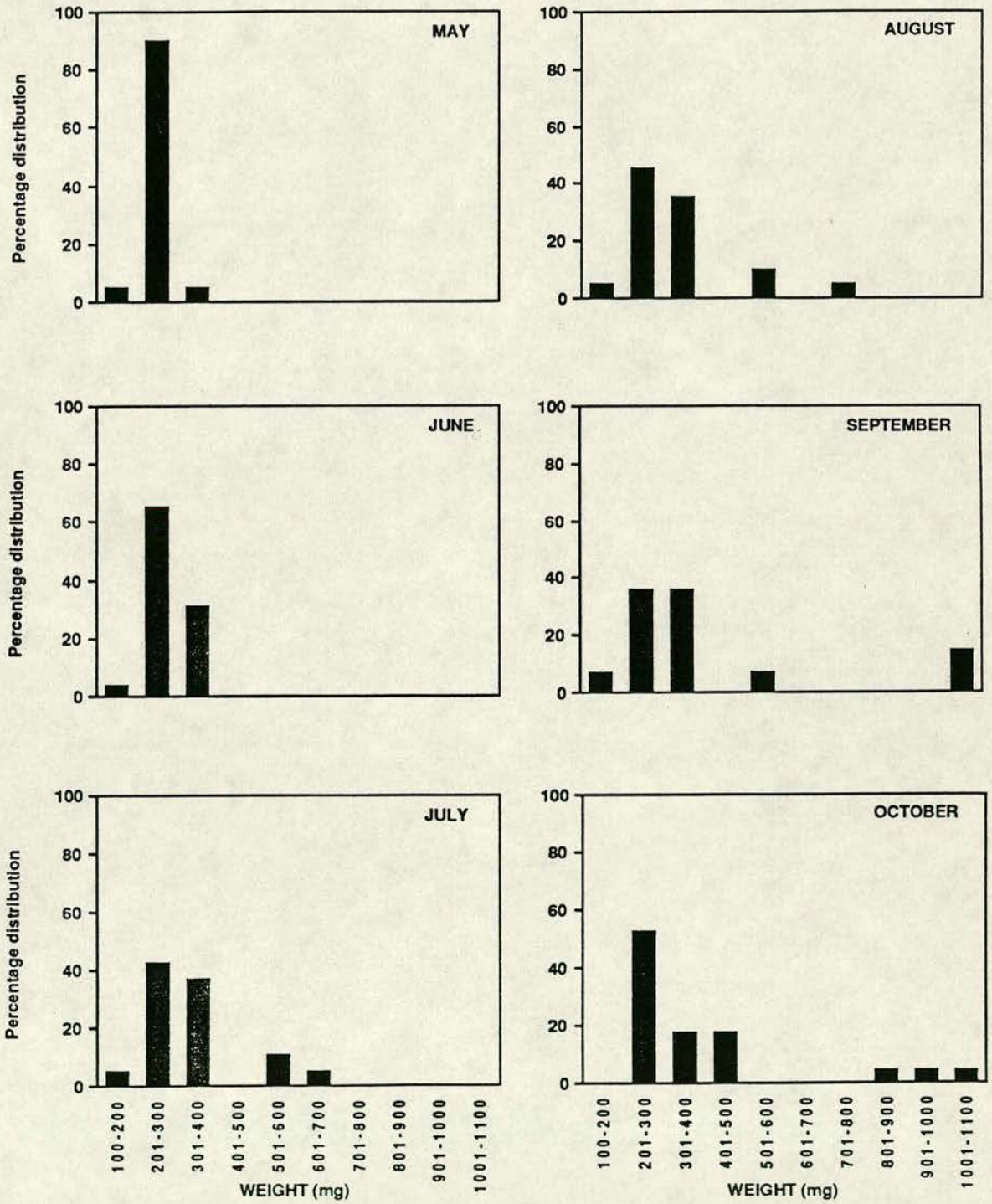


TABLE 6.2 Change in body composition of glass eels and elvers (n=sample size)

Date	Site	n	% water	% dry weight lipid	ash	protein
6/6/85	3	30	77.54	8.87	11.09	80.04
23/6/85	1	20	71.49	14.46	9.53	76.01
4/7/85	3	30	77.32	7.80	11.62	80.58
7/7/85	1	20	76.93	14.14	10.29	75.57
8/8/85	1	20	82.54	14.96	10.62	74.42
8/8/85	2	20	84.83	13.96	9.28	76.76
11/9/85	1	20	73.66	13.77	8.60	73.63
11/9/85	2	12	75.93	18.22	9.68	72.10
21/11/85	1	1	79.52	11.63	11.63	76.74
5/1/86	1	4	77.89	14.32	9.23	76.45
26/1/86	1	1	74.53	12.20	14.63	73.17
27/2/86	1	1	71.21	13.04	8.84	78.12
27/2/86	2	2	74.42	14.32	11.29	74.39
24/4/86	1	1	63.09	16.13	11.29	72.58
2/6/86	1	20	82.94	10.96	8.54	80.50
3/7/86	1	20	82.87	15.89	8.63	75.48
3/7/86	2	1	-	12.17	11.30	76.53

Recognition of year 0+ migrants was reliable during the transparent glass eel and pigmented stages up to VI A iv. Once the fully pigmented Stage VI B was reached otolith examination was required to differentiate elvers from slow growing year 1+ eels. The presence of yellow eels actively migrating up river with the glass eels was noted on 4/6/85 at Site 3, where three eels were caught with the glass eel sample, one of which had food in its stomach. All three were over 20 cm in length and over 5 years old. The majority of the glass eels continued their migration upriver in late July which may have been due to the reduction in flow rate (Table 2.1).

6.4 DISCUSSION

The first sighting of the glass eel migration in the River Almond occurred a month later in 1986 than 1985. This was also seen in the River Shannon for the same years (Moriarty, 1987b). The migration was not restricted by water temperature which had reached the reported minimum a month previously. However, continuous sampling would be required before one could be sure of the time of migration and the influence of the tides. All the glass eels in the River Almond samples were beyond Stage VI A iii_2, where ontogenic decrease in length and weight would be expected to have ceased. The migration may have been delayed in the North Sea for up to six months, that is, from December to May where there was probably little or no feeding (Tesch, 1973).

The upriver migration was also delayed at the weir (Site 3) for 2 to 3 months, where there was little evidence of feeding and no change in size, body composition or development of pigmentation (Tables 6.1 and 6.2). The latter is understood to be due to the lack of feeding where food items provide the materials for pigmentation, for example, lipid is required for yellow pigmentation (Tesch, 1973). The low energy requirement of the glass eels enables starvation to be withstood during this time, despite the high water temperatures which reached a maximum of 18 °C. Glass eels at Site 1 were feeding during this time and body composition was changing and pigmentation developing. There was a similar increase in lipid level with increasing size as seen in the eel population as a whole (Chapter 5).

There may be some density dependent factor which forces most of the glass eels to stay in the upriver migration (Moriarty, 1986) or their energy reserves may determine the length of time before feeding commences. Growth variation was high during the first year in freshwater and growth was rapid, which could be due to the suitability of the diet of mainly oligochaetes and chironomid larvae (Chapter 2).

6.5 SUMMARY

1. A continuous sampling method is required to secure more reliable data regarding the glass eel migration.

2. The weir between the sites delayed glass eel migration for 2 to 3 months.

3. Development of the glass eels was arrested during the delay at the weir which was made possible by their low energy requirement. Body composition did not alter, or pigmentation develop, until feeding commenced.

4. Growth variation was high during the first year in freshwater and increased when feeding commenced but decreased in the non-feeding glass eels at Site 3.

CHAPTER 7

TRANSFER OF GLASS EELS TO CULTURE

7.1 INTRODUCTION

The success of culture can be influenced by the methods used to capture, transport and transfer glass eels to the culture facility. Glass eels are caught commercially by various types of dip net or trap and are often held at collecting stations before transfer to culture facilities, where acclimatization to water of differing salinities and temperature may be necessary. The transfer process includes inducing the glass eels to feed, which usually involves first feeding with a natural diet before weaning onto a dry, compounded diet. This is considered to be most efficient (Kuhlmann and Koops, 1980; Knights, 1983) despite reports of successful first feeding directly onto dry diet (Kastelein, 1983). Adaptation to compounded food can involve high mortalities and can magnify the effects of growth variation (Wickins, 1983) which emphasizes the need for effective first feeding and weaning diets.

Water quality and feeding conditions vary in holding facilities at collecting stations, and the time that they are held depends upon the nature of the glass eel fishery and the market for glass eels. Culture success has been reported to be influenced by temporal and geographical factors, for example, *Anguilla japonica* (Matsui, 1952), and *Anguilla anguilla* glass eels caught in the Mediterranean were found to have faster growth rates than those caught in the Atlantic (Kuhlmann, 1974).

This chapter describes an experiment designed to examine the effect of an extended holding period under natural conditions on culture success, whilst assessing the effects of first feeding and weaning. Glass eels were transferred from the River Almond to culture in May, June and July and the effect of first feeding and weaning was assessed. The glass eel migration was first sighted in the River Almond on 5/5/85, but the passage upriver was delayed by a weir (Site 3) for 2 to 3 months. The development of the glass eels was delayed during this time, despite high water temperatures which reached 18 °C, and was probably due to feeding inactivity (Chapter 6).

7.2 METHODS (The culture system and holding, transfer, experimental and analytical methods are described in Chapter 1.2.2).

Fish

Glass eels were transferred to experimental culture facilities from the River Almond in May (15/5/85), June (6/6/85) and July (6/7/85). They were collected from the weir (Site 3) in a hand net, and transferred in buckets to a holding tank, where water at ambient temperature was replaced with water from the recirculation system over a period of two days, whilst feeding was witheld. Each month, 20 fish were randomly assigned to each of 3 x 10 l tanks in the recirculation system.

Initial individual fish length and weight were recorded. The glass eels were normally distributed with respect to weight at Site 3 in May, June and July (Figure 7.3) and were not significantly different from each other (Mann-Whitney *U*-test; $p<0.001$). Glass eels caught in May were graded to include fish between 200 to 300 mg for a transfer experiment before the availability of glass eels in June and July was realized. There were insufficient glass eels available for a similar grading in June and July and hence they remained ungraded. The length and weight of dead fish was recorded.

The significance of differences between the samples with respect to weight were tested by means of Mann-Whitney *U* -Tests; $p<0.05$, if not stated otherwise, (Siegel, 1956).

Diet

The glass eels were fed live tubifex at 20 % body weight per day (b.wt.day^{-1}). Tubifex is a common first feeding diet in the wild (Table 2.8) and culture (Kuhlmann, 1974). An extended period of first feeding with tubifex for 24 days was undertaken, to allow any differences due to time of transfer to develop, and to investigate the basic growth pattern. It was intended to continue the trial for a further 60 days for weaning onto a dry diet, and this was possible for the May transfer, but the experiment had to be stopped at day 36, before complete weaning of the June and July transfer,because of infection with *Ichthyophthirius multifiliis* (whitespot), which was first identified on 12/7/85.

The ration was adjusted every 12 days, after 11 days feeding and one days starvation, before growth measurement and recommencement of feeding on the 12th day. Weaning began after 24 days, where the proportion of tubifex was reduced by 25 % every 6 days, onto a dry diet ('Ewos Baker with Norsabel' 0, 1 and 2 crumb) at a feeding rate of 5 % b.wt.day^{-1}. The June and July transfer tanks were stopped at

day 36, after the first 12 day weaning period, and the May transfer was continued for a further 12 day weaning period (day 48) and 36 days on the dry diet (day 84).

7.3 RESULTS

7.3.1 Effect of transfer

The glass eels responded immediately to the live tubifex, and the ration was consumed within a few minutes in a bout of activity which resembled a feeding frenzy. Social interaction was high at feeding times and after two weeks the aggressive 'chase and bite' behaviour resulted in wounding. There was a tendency for larger eels to lie on the bottom of the tank, or curl around the inlet or outlet pipes, whilst smaller eels stayed in the water column.

The change in median weight of glass eels transferred in May, June and July is shown in Figure 7.1 and instantaneous growth rate (G) and percentage mortality (M%) in each 12 day period are shown in Table 7.1.

FIGURE 7.1 Change in median weight of glass eels transferred in May, June and July: Appendix 7.1 (mean of three replicates and their standard error)

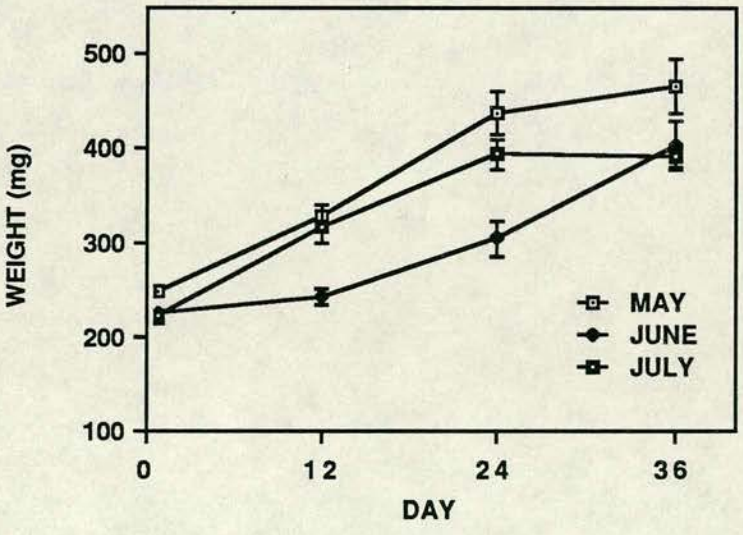


TABLE 7.1 Instantaneous growth rate (G) and mortality (M%), in each 12 day period, of glass eels transferred in May, June and July: Appendix 7.1

Month	Day	1-12	12-24	24-36	1-36
May	**G**	2.4	2.4	0.5	1.75
	M%	0	2	0	2
June	**G**	0.6	1.9	2.3	1.60
	M%	2	8	17	27
July	**G**	3.0	1.8	-0.06	1.57
	M%	50	3	0	53

Glass eels transferred in May (graded before the experiment) were significantly heavier than those transferred in June ($p<0.0009$) and July ($p<0.016$) whereas the June and July transfers (not graded) were not significantly different from each other. After 36 days the May transfer was significantly heavier than June ($p<0.0136$) and July ($p<0.0022$), whereas June and July were not significantly different. Graded fish of median weight 0.250 g (May) remained distinct from fish of median weights 0.227 g (June) and 0.222 g (July) with respect to growth.

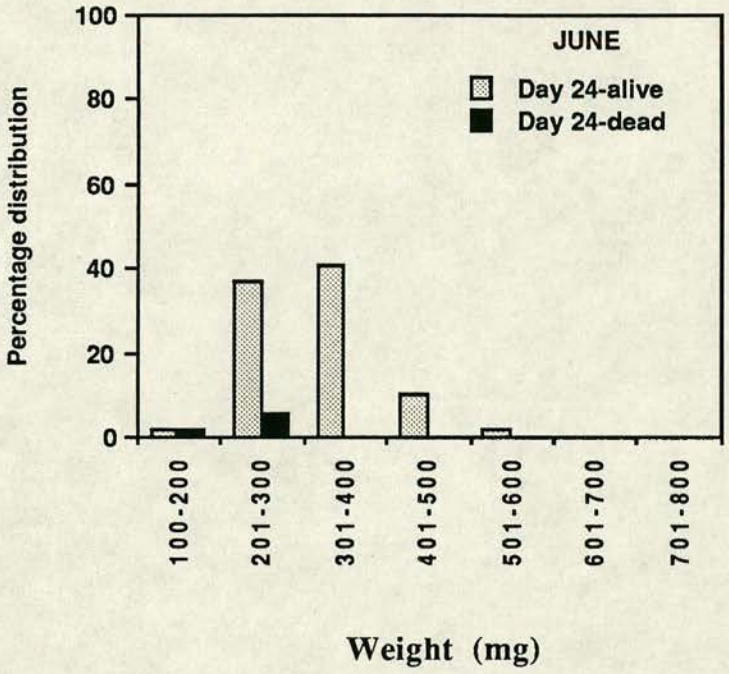
The survival rate of the glass eels transferred up to day 36 varied from 98 % in May to 73 % and 47 % in June and July respectively. The likely reason for the high mortality was infection with *Ichthyophthirius multifiliis* (whitespot) which was probably introduced into the recirculation system with glass eels transferred in July, despite preventative treatment (Chapter 1). The parasite was also present in samples of wild eels (Chapter 4.2). Whitespot was probably responsible for mortality of 50 % of the glass eels transferred in July, which died within the first 12 days, with a further 3 % before the end of the experiment, and 17% of the June transfer. However, 10% of the June transfer and 2% of the May transfer died before whitespot was introduced to the system, and this mortality was probably due to aggressive chase and bite behaviour. The dead fish often had tail damage, and were generally smaller, as shown in Figure 7.2.

G over the 36 day period was 1.75 % for May and 1.60% and 1.57 % for June and July respectively. However, differential survival of fish of different sizes had the effect of increasing the median weight and hence G for the 12 day period (3.0 %) in the July transfer. A similar effect was seen at the end of the trial in the June transfer

(2.3 %). G was low at day 12 in the June transfer, that is 0.6 % compared with 2.4 % for May, but had increased to 1.9 % by day 24. The high growth rates achieved by all transfers by day 24 were reduced markedly during weaning, that is, 0.5 % and -0.06 % for the 12 day weaning period for May and July transfers respectively. The growth rate during this period for the June transfer (2.3 %) was erroneously high due to mortality of smaller fish.

Growth variation was greatest in the May transfer, where the coefficient of variation (CV%) had risen to 41.1 % in one replicate tank (Table 7.2). CV% was virtually constant during the first 12 days in May and June, and the high mortality in the July transfer during this time precludes comparison. The divergence in growth, over 36 days, from the initial normal weight distribution is shown in Figure 7.3.

FIGURE 7.2 The effect of weight of individual fish on mortality



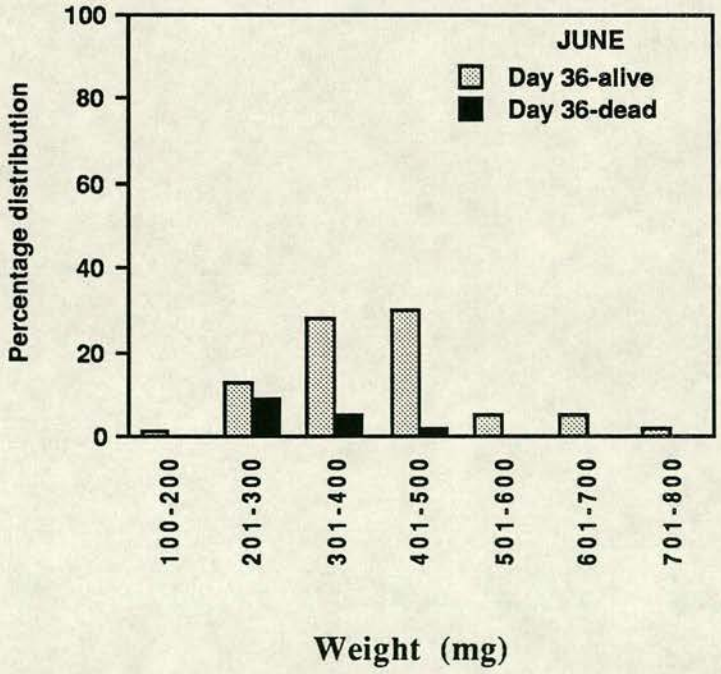
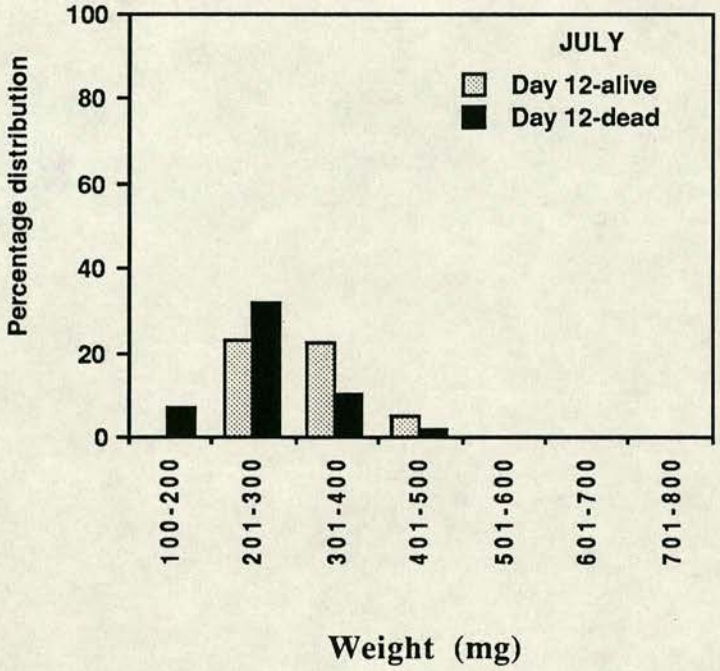


FIGURE 7.2 continued. The effect of weight of individual fish on mortality



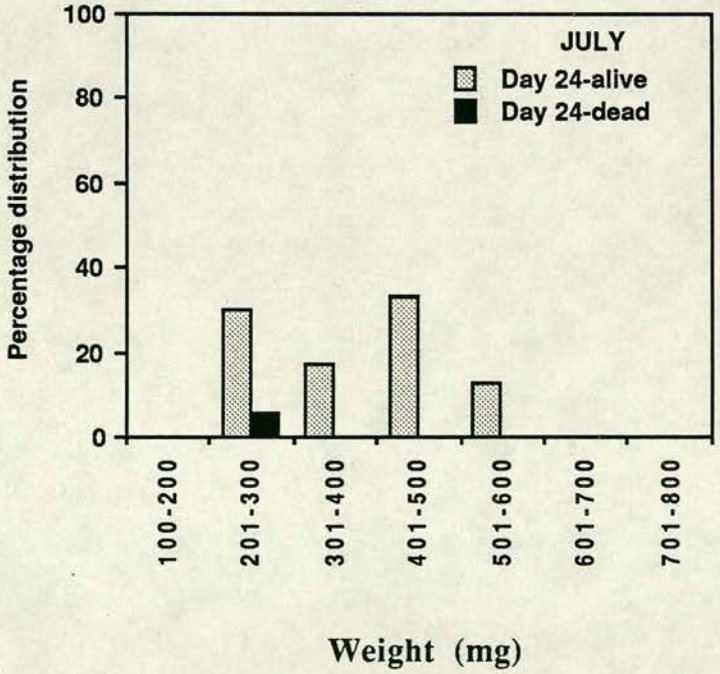
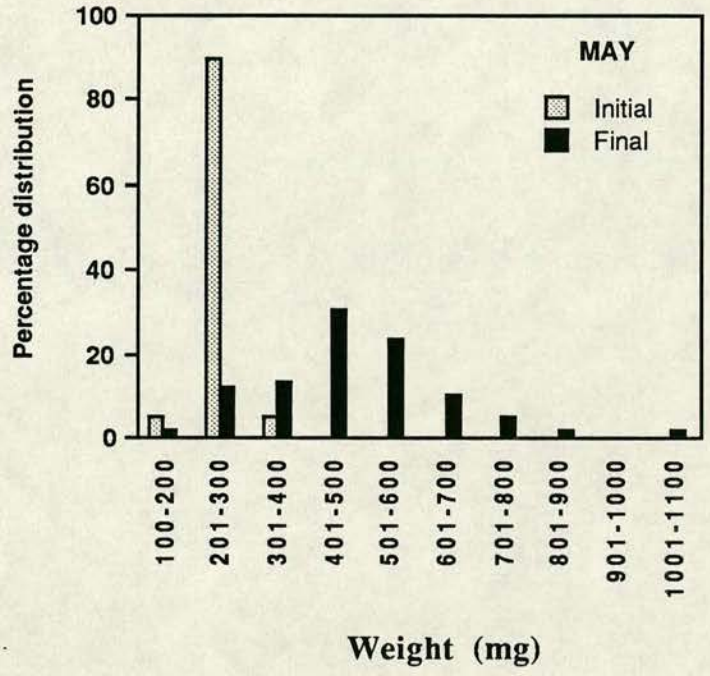


FIGURE 7.3 Change in weight distribution of glass eels in culture



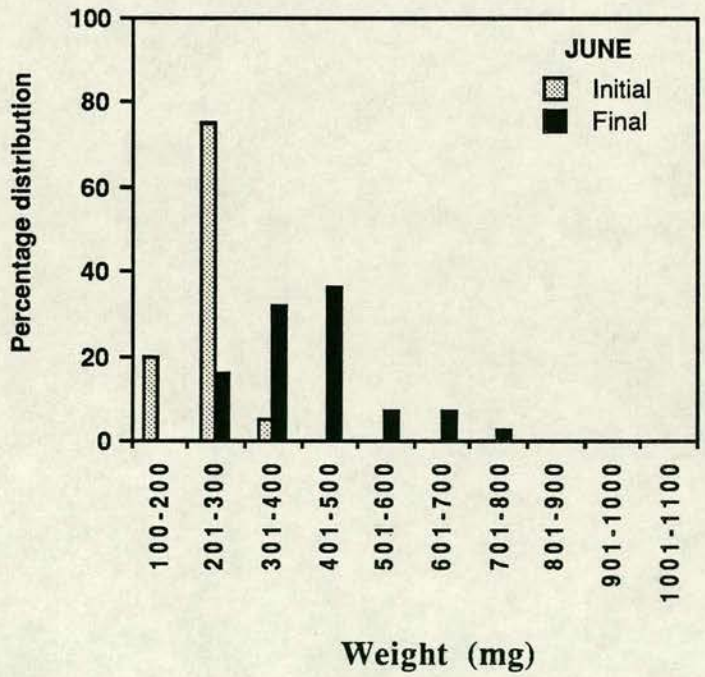
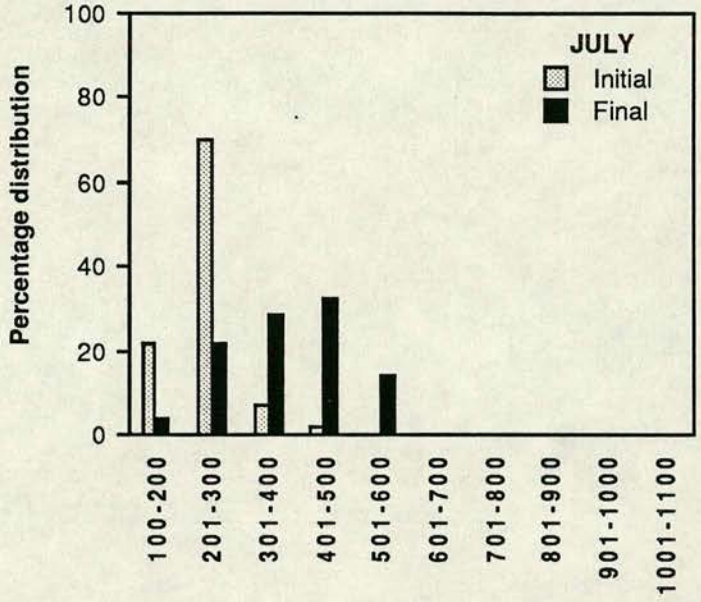


FIGURE 7.3 continued. Change in weight distribution of glass eels in culture



7.3.2 Effect of weaning

Glass eels did not accept dry food readily. They nudged the food with their snouts against the sides of the tank or outlet, before taking it into their mouths, often to spit it out before swallowing. Glass eels transferred in May were weaned onto dry diet by day 48, and feeding continued until day 84.

CV% of glass eels transferred in May, June and July are shown in Table 7.2 for each 12 day period, G, M% and the percentage increase in coefficient of variation (CV*) for each 12 day period for the May replicates are shown in Table 7.3.

G was high in all replicates on live tubifex and was over 2 % day^{-1} up to day 24. G dropped below 1 % during weaning to become negative after the first 12 days on a wholly dry diet (days 48 to 60). G was more erratic on dry diet and interpretation of the data was complicated by mortality. There were fish which had lost weight, maintained their initial size or gained weight at varying rates up to eight times their initial weight, or 2.5 % day^{-1} over the 84 day experimental period.

CV* remained virtually constant during the first 12 days on live tubifex in all

replicates, to increase by over 60 %, between days 12 to 24, in replicates 1 and 3 and over 94 % in replicate 2. The increase in CV* from one 12 day period to the next was very similar in replicates 1 and 3, except between days 36 to 48, where 3 fish died in replicate 3.

M% was highest in replicate 2, where the dominant fish weighed over 1 g by day 36, and aggressive chase and bite behaviour was the probable cause of death of all but two fish by the end of the experiment.

TABLE 7.2 Coefficient of variation of glass eels transferred in May, June and July : Appendix 7.1 (r=replicate)

	r	Day 1	12	24	36	48	60	72	84
May	**1**	11.3	11.1	17.9	24.2	27.6	36.3	48.4	61.3
	2	13.9	14.1	27.4	41.1	56.4	72.7	94.9	-
	3	14.2	14.4	23.5	32.0	25.9	33.3	45.3	58.1
June	**1**	16.1	16.7	18.9	22.9	-	-	-	-
	2	16.7	17.3	24.4	26.8	-	-	-	-
	3	13.2	13.3	21.0	24.7	-	-	-	-
July	**1**	19.0	14.2	21.2	19.9	-	-	-	-
	2	23.0	17.0	17.8	17.4	-	-	-	-
	3	16.5	20.6	30.3	33.2	-	-	-	-

TABLE 7.3 Instantaneous growth rate (G), percentage increase in coefficient of variation (CV*) and mortality (M%) of glass eels transferred to culture in May : Appendix 7.1 (r = replicate)

r	Days	1-12	12-24	24-36	36-48	48-60	60-72	72-84	1-84
1	G	2.71	2.30	0.88	0.66	-0.80	1.72	0.08	1.08
	CV*	-	61	35	14	32	33	27	443
	M%	-	-	-	-	-	-	10	10
2	G	1.88	2.06	0.37	1.35	-1.24	-0.71	-	-
	CV*	-	94	50	37	29	31	-	-
	M%	-	5	-	60	-	25	-	90
3	G	2.43	2.71	0.32	0.42	-0.34	0.42	0.52	0.93
	CV*	-	63	36	-	29	36	28	309
	M%	-	-	-	15	5	-	5	25

7.4 DISCUSSION

It was difficult to compare the growth performance of glass eels transferred to culture in May, June and July because of infection with whitespot, which caused high mortality in the June and July transfers. There was a lag phase in the growth of the June transfer, compared with the May transfer, during the first 12 day period. This may have been a result of the delay in development in the river, but whether this occurred in the July transfer was masked by high mortality.

Glass eels probably have the same genetic potential for growth despite an initial size differential, which is thought to reflect migration history rather than genetically poor and good growers. Wickins (1987) found no significant difference between the growth of elvers graded into different size groups 3 days after capture, but when graded 30 days after capture, growth was significantly different. Here, the smaller groups grew less well, which was thought to be in response to suppression during the holding period, which was not reversible after grading. The growth of the different groups remained distinct from each other, which was also seen in glass eels transferred in May, compared with June and July (Figure 7.1). It appears that delay in development under natural conditions has a similar effect as that in holding facilities

in culture, where some selection process may be operating.

Once feeding begins, in the wild and in culture, variation in the growth rate of individual fish becomes apparent. Similar extremes of growth variation are seen in the wild during year 0+ (Figure 6.1) and in culture over a few months (Figure 7.3). The rate of development of the growth differential is increased in culture where once an advantage is gained by a particular individual, the growth differential can increase at a rapid rate, often leading to mortality of the smaller fish. Differential growth in culture has been related to the effect that agonistic behaviour has on appetite and feeding (Seymour, 1983).

The initial cause of growth inequality in size-matched fish in culture is thought to be determined by individual genetic differences in aggression, sex or prior social experience (Knights, 1985). This has also been described as due to differences in vigour or susceptibility to stress, which develop as a result of some 'genetic predisposition' (Kuhlmann, 1974). Growth variation increased during the high growth rate obtained on a tubifex diet, and during weaning, when growth rate was reduced which was probably due to the stress caused by the changing conditions. Growth inequality is maintained by the development of a hierarchy where increased activity is forced upon subordinates (Knights, 1985) which receive less food and are subjected to more stress (Koebele, 1985). Differences in vigour are also thought to be responsible for changes in hierarchical positions following a new stressor, which is independent of initial size and hierarchical position. This new stressor could be a change in almost any factor including grading, weighing, diet or isolation (Cripps, 1983; Wickins, 1987). Once a hierarchy develops the large fish can grow at a faster rate than the smaller, that is, not just a function of size otherwise the rate would be the same (Purdom, 1974). The smaller fish are suppressed by the larger which in turn may actually be stimulated to increase their growth rate and are known as 'shooters'. Permanency of stunting is not absolute as fish growing slowly within a population may increase their growth rate once isolated, indicating that the suppression has been removed (Wickins, 1985).

Mortality can be due to aggressive behaviour of the larger fish or to the reduced fitness of the smaller fish which renders them more susceptible to disease or other culture stresses. The growth differential of a single year class in the wild is high but it is unlikely that cannibalism within a single year class would account for mortality (Chapters 2 and 3), unlike the situation in culture once fish have reached a growth differential of x1.5 (Knights, 1985). Cannibalism was not observed in this experiment where mortality was due to the secondary effects of aggressive behaviour, that is an osmotic imbalance caused by wounding during the chase and bite behaviour, and infection with whitespot. The smaller fish were more susceptible which tends to

indicate that mortality was due to the combined effect of aggression and infection (Figure 7.2). Starvation in itself may not be a cause of mortality in the wild or culture until development is triggered by feeding, considering the eels' physiological capacity for delayed development.

The physiological capacity of glass eels for delayed development allows their capture and holding in collecting stations for transportation for fisheries and aquaculture stocking purposes world-wide although this may affect the growth potential. Problems with maintenance of water quality and the effects of growth variation, are minimised by withholding food and lowering temperature during this time. Losses incurred during weaning could be kept to a minimum by reducing other potential stressors during this critical period, for example, grading between first feeding and weaning would reduce social interaction, although grading itself is stressful. Thus, a balance has to be made if the aim of culture, of optimum growth of the whole population, is to be attained. The effect of diet and social interaction on growth rate and growth variation in culture populations is discussed in the following chapter, where an attempt is made to put the more fundamental elements involved in variation, both genetic and environmental, into context.

7.5 SUMMARY

1. *Ichthyophthirius multifiliis* (whitespot) was probably introduced to the recirculation system with glass eels in July, and resulted in heavy mortality of the less developed glass eels, that is, those transferred in June and July. Hence, it was difficult to compare growth performances and evaluate possible effects of delay in development. However, the June transfer took longer to acclimatize to culture conditions than the May transfer and were more susceptible to disease.

2. Holding of glass eels under natural conditions and in holding facilities in culture appears to have similar effects on future growth. Differences in 'vigour' develop before feeding and size differential which result in growth depensation when feeding commences.

3. A size and dominance hierarchy develop in a newly formed population within approximately two weeks.

4. Growth variation is more extreme under culture conditions than in the wild and is related to the effect that agonistic behaviour has on appetite and feeding. Growth inequality is maintained by the development of a hierarchy, where more activity is forced on subordinates which receive less food and are subjected to more stress, which reduces appetite and resistance to disease.

5. High mortality resulted from the secondary effects of chase and bite behaviour which encouraged secondary infections and osmotic imbalance, and increased susceptibility to whitespot in the smaller individuals. Starvation may not be a major cause of mortality until development is triggered by feeding, due to the eels' physiological capacity for delayed development.

6. Growth variation was high with rapid growth rate on a live tubifex diet and during weaning onto dry diet, where growth rate was reduced and stress was likely to be higher on the dry diet.

CHAPTER 8

APPLICATION OF NATURAL CONDITIONS TO CULTURE

8.1 INTRODUCTION

The growth differential in a population of elvers is more extreme under culture conditions than in the wild (Chapter 7). The extent that this is due to environmental factors will determine whether this phenomenon can be artificially controlled, in order to optimise growth of the whole population, rather than create an avenue of opportunity for a few fish.

Some of the environmental factors affecting growth in culture, that may differ from natural conditions are temperature, water quality, lighting, food quantity and quality and social interaction. The optimum temperature for culture is likely to fall within the range of 23 to 27 °C (Kuhlmann, 1974) whereas the highest recorded temperature in the River Almond was 18.5 °C (Chapter 2). Temperature has been found to increase the rate but not the extent, of the development of the growth differential (Kuhlmann, 1974). Water quality, if maintained to a high standard, and lighting, if it approximates natural conditions, will probably not affect growth variation (Chapter 2). Temperature and light were held constant during the growth experiments.

This chapter describes two experiments designed to examine the influence of diet and social interaction on growth variation in culture, with reference to the degree that each factor in culture departs from the situation in the wild. In ***Experiment 8.1*** the physical properties of tubifex are changed to approximate different properties of a commercial dry diet, in order to determine its effect on growth variation and acceptance by eels. In ***Experiment 8.2*** the effect of social interaction on growth variation is tested by providing a physical substrate, which approximates the natural living conditions of eels and allows the eels to be solitary without an unnatural situation of total isolation.

8.1.1 ***Experiment 8.1*** A suitable diet is extremely important in culture, where the aim is to maximise growth of the whole population, at least cost, with minimum wastage and fouling of the water. Depending upon the type and quality of diet the mean weight of populations of cultured eels can differ by 100 %, reaching 210 g versus 420 g after 1.5 years, from an initial weight of 40 g (Koops and Kuhlmann, 1981). Various unprocessed foods have been used in the culture of the Japanese eel *Anguilla japonica* including silkworms, fish and offal, which have now largely been replaced by fishmeal-based paste diets (Usui, 1974; Gousset, 1988). For *Anguilla anguilla* in warm water effluents, modern particulate diets are preferred because of their increased stability in water and ease of storage, handling and dispensing (Appelbaum, 1980). However, dry diets are not readily accepted by glass eels and elvers and are not ideal for eels once they are weaned (Kuhlmann and Koops, 1980).

Formulation of an acceptable and nutritionally balanced diet and the feeding techniques used to dispense it, requires a knowledge of the sensory and motor capabilities, and behavioural attributes of the culture species, at each stage of development, and for each culture method adopted. A diet must satisfy both physical and chemical criteria to attract fish to the food and incite feeding, ingestion and continuation of feeding. These criteria include : appearance (size, shape and colour); texture (roughness, hardness and wetness) and smell and taste (Mackie and Mitchell, 1985). The efficiency of location, capture success and ease of ingestion have been shown to be directly related to growth in Atlantic salmon *Salmo salar* (Wankowski and Thorpe, 1979), where bioenergetic and optimal foraging theory have been implicated (Knights, 1984) and (Krebs, 1978).

8.1.2 ***Experiment 8.2*** Glass eels and elvers lead a shoaling semi-pelagic existence during the elver migration, when population densities can be very high, but become more solitary and territorial on progression to a more benthic life. Little or no feeding occurs at the glass eel stage, and while actively migrating (Tesch, 1973), and development is delayed until they join the benthos (Chapter 6). Under culture conditions, these naturally aggressive fish are crowded together in a situation where competition for suitable space on the bottom of the tank is high (Knights, 1984) and where there will probably be competition for food, despite the high feeding rates (Purdom, 1974). Growth variability of non-shoaling culture species of marine flatfish has been shown to decrease on isolation (Purdom, 1974). Conversely some shoaling species are restless and excitable when isolated from like members of a similar size, and normal growth is interrupted, for example, the herring *Clupea harengus* (Blaxter and Holliday, 1963). Newly caught elvers have shown enhanced growth variability on

isolation, whereas growth variation of older elvers was generally depressed by isolation (Wickins, 1985). This author interpreted it as being due to the isolation of normally gregarious elvers which were still leading a shoaling existence however communal living may also be required to elicit a feeding response (Knights, 1985).

8.2 METHODS (The culture system and holding, transfer, experimental and analytical methods are described in Chapter 1.2.2).

Fish

Elvers were obtained from 'Bristol Channel Fisheries', River Severn, UK. After one week in the quarantine tank they were transferred to the culture system, having been graded to reduce the initial size variation and subsequent growth variation (Jobling, 1982).

Experiment 8.1 Elvers were graded to include fish from 200 to 300 mg, and 20 fish were randomly assigned to 12 x 40 l tanks, in 4 different treatments. Dead fish were replaced during the first week.

Experiment 8.2 Elvers were graded into five, 50 mg size groups, from 150 to 399 mg, and elvers from each size group were randomly assigned to 2 tanks, 12 fish per 40 l tank. Of the two sets of five size groups, one set had gravel added to the tanks, and one set remained gravel free, as the control.

Diet

Live tubifex were obtained from commercial sources each week and kept in holding facilities (Chapter 1). Samples of tubifex were taken each week for proximate analysis (Chapter 5.2) and mean values for body composition were:- 80.08 % moisture; and 22.08 % lipid; 3.46 % ash and 74.46 % protein on a dry weight basis.

Experiment 8.1 Tubifex was fed in four forms, that is, live, chopped, freshly dead and dried at a feeding rate of 20% body weight per day (b.wt.day^{-1}) wet weight and 5 % b.wt.day^{-1} dry weight. Food was offered twice a day for 6 days, with 1 days starvation, before measurement of weight (to the nearest 0.001 g) and length (to the nearest 0.1 cm) on day 8, when the feeding ration was adjusted. The regime was continued for 28 days.

There were on average 600 worms per gram of live tubifex. Tubifex were freshly chopped before each feed, which was standardized by number of cuts with a scalpel blade per gram of tubifex spread in a petri dish, on average each worm was cut into three pieces which were still moving on feeding. Tubifex were killed by immersing in liquid nitrogen for 20 seconds before each feed, which was preferred to

other killing methods, such as dipping in boiling water or freezing, because it did not alter the appearance or rupture the skin of the worm. Dried tubifex was provided by freeze-drying a sample of each weekly supply of live tubifex, in a model EF2 Edwards Freeze Drier, for one day. The particle number per gram of the different forms of tubifex, expressed as a ratio of live tubifex, were: chopped (3:1); freshly dead (1:1) and dried (0.2:1).

Experiment 8.2 Glass eels were fed live tubifex twice a day at a level of 20 % b.wt day^{-1} for 6 days, starved on day 7 and individually weighed on day 8, when the feeding ration was adjusted and the regime continued for 5 weeks.

Substrate

Experiment 8.2 The substrate was provided by inserting three litres of washed gravel (Dorset pea gravel) into the 5 treatment tanks whilst the 5 control tanks remained gravel free. The fish in the gravel substrate were separated by hand from the gravel at each weighing interval and the faeces, which had collected in the gravel, were removed. There were also small amounts of tubifex remaining in the gravel, and at the end of the trial the fish in the gravel tanks were re-weighed the following day, after the gravel had been washed, to check that any tubifex remaining in the gravel were a negligible food source. It was found that starvation had been effective, as there was no significant difference in the weight of fish on the two occasions (Mann Whitney *U*-test; $p<0.001$).

Disease control

Experiment 8.1 Initial treatment with formalin and malachite green (Chapter 1).

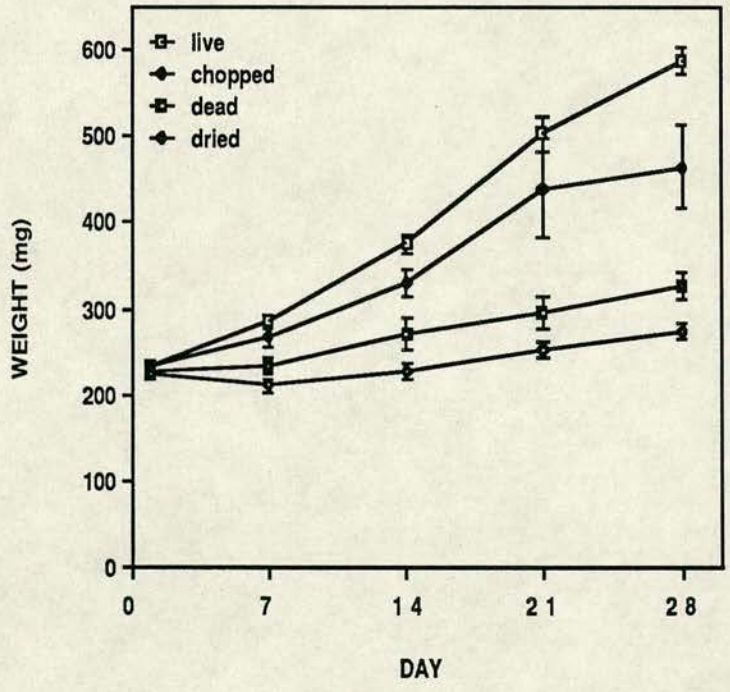
Experiment 8.2 Initial treatment with formalin and malachite green and in addition, a malachite green based prophylactic (W.S.3) was added to the water in the recirculation system at weekly intervals to control *Ichthyophthirius multifiliis* (whitespot).

8.3 RESULTS

8.3.1 ***Experiment 8.1*** There was no food remaining in the tanks from the previous feeding-time, except for strands of dried tubifex, which were caught in the outlet screens. There was a problem with infection with *Ichthyophthirius multifiliis* (whitespot) despite initial preventative treatment and mortality was high. Dead fish were usually the smaller individuals and showed signs of tail damage due to chase and bite behaviour.

The changes in median weight of elvers over the trial period on the different treatments are shown in Figure 8.1. Initial median weights of the tank populations were not significantly different. After 28 days fish on live tubifex were significantly heavier than the other treatments, the chopped tubifex treatment were significantly heavier than the dead and dried tubifex treatments and there was a significant difference between the dead and dried tubifex treatments, Mann Whitney *U* -test; $p<0.05$.

FIGURE 8.1 ***(Experiment 8.1)*** **Change in median weight of elvers on a diet of tubifex in different physical form : Appendix 8.1 (mean of 3 replicates and their standard error)**



Instantaneous growth rate (G), coefficient of variation (CV%) and survival (S%) over the experimental period are shown in Table 8.1. S% ranged from 20 to 80 % among the replicates and was highest on the live tubifex, less on the chopped and dead tubifex and lowest on the dried tubifex. Mean values of the replicates for G varied from 0.72 to 3.29 % and were particularly high on the live tubifex, that is, above the expected maximal value of 3 % day^{-1} (Seymour, 1984). G on the chopped tubifex was nearly twice that on the dead tubifex and G on the dried tubifex did not

exceed 0.84 %. G during the first 7 days was lower than in succeeding weeks in all treatments but was particularly low on the dead tubifex and dried tubifex, that is, 0.50 % and -0.86 % respectively (Appendix 8.1). CV% ranged from 11.3 to 35.8 % among the replicates and was lowest where survival was lowest, which was probably due to mortality of the smaller fish. CV% was highest on the live tubifex diet and lowest on the chopped tubifex, but was very variable between replicates in all treatments.

TABLE 8.1 ***(Experiment 8.1)*** **Instantaneous growth rate (G), coefficient of variation (CV%) and survival (S%) of elvers on a diet of tubifex in different physical form : Appendix 8.1** **(t=number of days, r=replicate)**

Diet	r	G(t=28)	CV%	S%
Live	**1**	3.40	28.0	50
	2	3.33	34.6	55
	3	3.13	25.9	75
Chopped	**1**	1.78	12.9	40
	2	2.54	26.1	80
	3	2.96	18.7	25
Dead	**1**	1.65	19.3	35
	2	1.11	35.8	55
	3	1.20	22.6	55
Dried	**1**	0.84	22.7	45
	2	0.56	11.3	20
	3	0.75	34.2	50

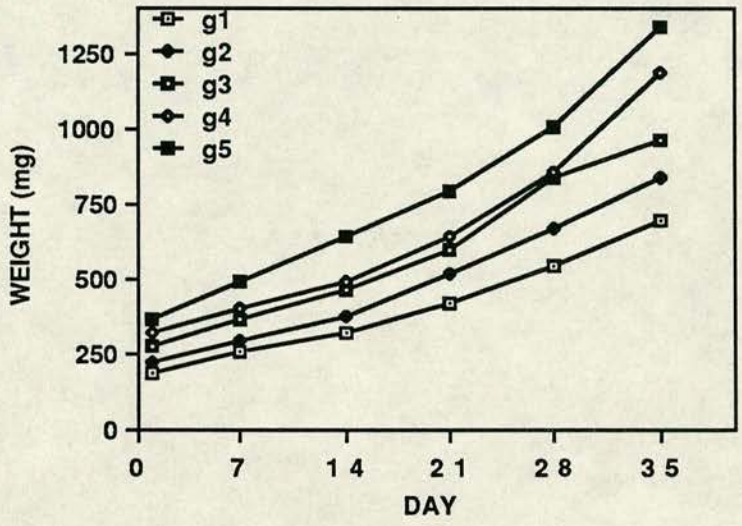
8.3.2 ***Experiment 8.2*** Elvers remained buried in the gravel, with just the top of their heads protruding from it, except for the few minutes at feeding time, during which aggressive chase and bite behaviour occurred. In the non-gravel tanks social interaction was observed throughout the day and chase and bite behaviour was common.

The change in median weight of each size group of elvers in the gravel and non-gravel tanks over the trial period is shown in Figure 8.2. There was no significant difference between the initial weight of the replicates in each size group. Each size group in the gravel was significantly heavier than its replicate in the non-gravel at the end of the trial (Mann Whitney *U*-test; $p<0.001$). Final median weight was dependent upon initial median weight and this was most clearly demonstrated in the gravel tanks.

Instantaneous growth rate (G), coefficient of variation (CV%) and percentage survival of each size group in the gravel and non-gravel tanks over the trial period are shown in Table 8.2. G was high and very even among the replicates in the gravel tanks, although size group 3, at 3.55 %, was slightly less than the other size groups, at 3.72 ± 0.1 %. G of replicates in the non-gravel was more than 2 % day^{-1} over the trial period, except for size group 3, at 1.10 %. CV% increased in all replicates over the trial period, where values ranged from 22.5 to 52.1%. CV% did not vary consistently with treatment , or size of fish, that is, CV% was higher at the end of the trial in size group 3 and 4 in the gravel than the non-gravel and lowest in size groups 1, 2 and 5 in the gravel. S% was 100 % in the gravel and 85 % in the non-gravel. Loss of one fish from size group 2 in the gravel was due to its escape during weighing whereas losses in the non-gravel were due to mortality of the smaller fish. All the dead fish had tail damage inflicted through aggressive chase and bite behaviour.

FIGURE 8.2 ***(Experiment 8.2)*** **Change in median weight (mg) of different size groups of elvers in in gravel (g) and non-gravel (ng) : Appendix 8.2**

(1=150-199mg; 2=200-249mg; 3=250-299mg; 4=300-349mg; 5=350-399mg)



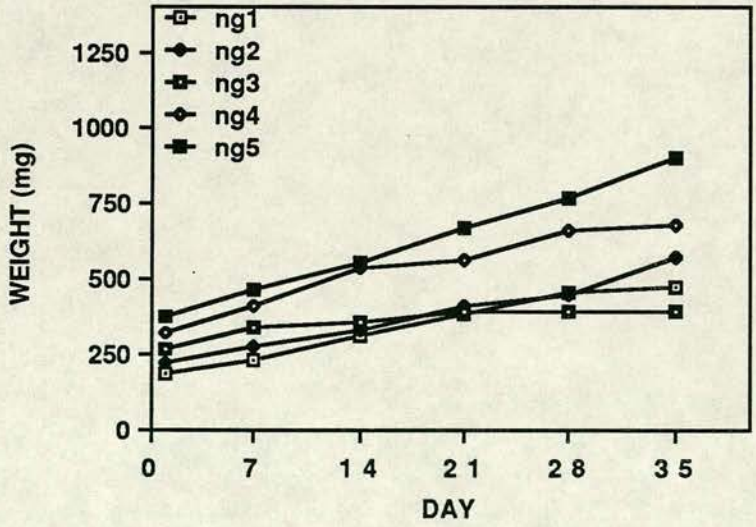


TABLE 8.2 ***(Experiment 8.2)*** **Instantaneous growth rate (G), coefficient of variation (CV%) and survival (S%) of elvers in gravel(g) and non-gravel (ng) : Appendix 8.2 (t=number of days, 1=150-199mg, 2=200-249mg, 3=250-299mg, 4=300-349mg, 5=350-399mg)**

Size	G(t=35)		CV%		S%	
	g	ng	g	ng	g	ng
1	3.73	2.59	22.5	29.8	100	92
2	3.73	2.72	27.1	52.1	100	67
3	3.55	1.10	30.4	28.8	100	75
4	3.71	2.15	38.4	31.1	100	100
5	3.71	2.51	30.6	33.4	100	92

8.4 DISCUSSION

8.4.1 ***Experiment 8.1*** The absolute nutritional value of the treatment diets probably did not vary as the same food was presented in different forms. There may have been more wastage with the dried tubifex, which floated, and was difficult for the elvers to eat, as their feeding behaviour involved nudging food items against the bottom, or sides of the tank, before taking it into the mouth (Knights, 1983). There may also have been some wastage with the chopped tubifex through leaching of nutrients, but otherwise all the food given was consumed. Growth was significantly affected by the physical form of tubifex, and was highest in diets which most closely resembled natural food, and was probably related to the bioenergetic costs of location, capture and ingestion.

Growth was lowest in all treatments during the first week which may have been due to the new conditions created by setting-up the trial (Kuhlmann, 1974), which would include learning to respond to a new food. Growth was particularly low in the dead and dried tubifex treatments, that is, G of 0.50 % and -0.86 % respectively (Appendix 8.1).

The size of the food item taken is determined by the mouth width of the eel. In the River Almond eel population there was a significant relationship between the largest width of prey organism and interpupillary distance, which is related to mouth width, (Törlitz, 1922), $r = 52$; $p<0.001$ (Figure 2.12). The eel has a small mouth

when compared with other cultured fish, such as the salmonids and cyprinids. When food items are too large to be taken whole, pieces are torn off into manageable sized portions, perhaps after soaking and softening, which leads to wastage and pollution. The length of the food item is not as limiting and can be quite long, especially when sucked into the mouth. However, with compounded dry feed more rejections and disintegration of pellets occurs if they are much longer than their diameter (Knights, 1985) and hence, optimum length of the food item will be influenced by other properties, such as texture. Handling time of the food has an energetic cost which increases if food has to be chewed rather than swallowed. Food diameter corresponding to mouth width has been found with soft paste balls (Knights, 1983). The optimum width of a dry food particle is reported to be about 0.4 - 0.6 of mouth width (Knights, 1983) and thus, is severely limited by the texture of the food item. Once in the mouth food items are further tested, and taste and texture determine whether they are rejected or swallowed. Food can be rejected after swallowing, for example, an eel was observed by the author to regurgitate after swallowing, a large number of the lumbricid worm *Eisenia foetida*. This species was also found to be unpalatable to rainbow trout *Salmo gairdneri* (Tacon *et al.*, 1983).

Smell, which in the case of fish should be termed long-range chemical attraction (Mackie and Mitchell, 1985), and taste are used by fish to detect food at a distance and taste buds in the mouth and pharynx monitor the taste and determine whether the food is swallowed. Feeding stimulants, that is, attractants and incitants, (Lindstedt, 1971 in Mackie and Mitchell, 1985), are picked-up as odours emitted from the food organism. The response of particular fish species to artificial feeding stimulants is related to the natural food organisms and probably taxonomy, at below family level. The natural food organisms of both *Anguilla anguilla* and *A. japonica* include Crustacea, Mollusca, worms and small fish, all of which are a rich source of L-amino acids, which are the major food attractants for these two species (Mackie and Mitchell, 1983; Takeda *et al.*, 1984). Different chemicals, or concentrations of the same chemical, may have attractant and palatable properties inciting the olfactory and gustatory senses.

The eel is predominantly a nocturnal feeder in the wild (Tesch, 1973) which is related to its increased activity during darkness (Kuhlmann, 1974) and therefore, it is likely that chemosensory perception is strong. In feeding experiments conducted in light, fingerling eels used their chemical senses to search for food, where taste receptors on the flanks of the fish appeared to be involved and touch alone was not responsible (Mackie and Mitchell, 1983). However, some taste fibres also possess mechanoreceptor activity (Bardach and Villars, 1974 in Knights, 1985).

Vision becomes more important in food location by eels over 15 g although they still possess good chemosensory and mechanosensory abilities (Knights, 1982) and in the wild, where they become more piscivorous above 50 cms (Moriarty, 1975). Elvers and fingerling eels locate food on the bottom of the tank with their nose or head, as far back as the pectoral fins, and 'nudge' food by pushing with their nose (Knights, 1983).

Elvers may be inherently responsive to live tubifex and suited to its physical and chemical properties where little or no learning is required. The width and length of the food item is not limiting because of its soft texture and the elvers' ability to suck food into the mouth through inertial suction, an ability shared by most teleosts, involving rapid expansion of the orobranchial cavity to draw in water plus food from a distance (Hyatt, 1979). Live tubifex also has chemical attractant properties, although incitant properties are not necessarily as strong (Mackie and Mitchell, 1983).

Growth was higher with the chopped rather than the dead tubifex, despite the leaching of nutrients. The attractant properties of tubifex may be increased with chopping. The particle number per gram was increased, which would extend the handling time, although the chance of hyperphagia by a few dominant fish would be reduced. The subordinate fish would have more chance to feed and might explain the lower CV% with chopped tubifex. Growth was low on dried and dead tubifex. The dried tubifex was physically unsuitable, and there was some wastage, and the dead tubifex was a poor diet, despite the more suitable physical properties and lack of wastage. These results indicate the suitability of live food for eels.

8.4.2 ***Experiment 8.2*** Culture conditions encourage aggressive chase and bite behaviour in eels. Mortality is increased due to wounds sustained from bites and susceptibility to disease, while growth rate is decreased, due to energy expended in increased activity. A gravel substrate reduced social interaction and aggressive behaviour to a few minutes at feeding time, and prevented mortality and growth reduction, but had no influence on growth variation. Growth variation was considerable in both gravel and non-gravel treatments, despite the different levels of social interaction, and would appear to have some genetic origin (Matsui, 1952; Kuhlmann, 1974).

In both experiments, culture under more natural conditions was shown to enhance survival and growth rate, but to have little or no effect in reducing growth variation. The increased rate of development of a growth differential in culture, compared with the wild, is probably due to the increased temperature and feeding rate. Until breeding in captivity is possible, progress in culture maybe limited to the

provision of conditions for maximum survival and growth rate, with growth variation being continually controlled by frequent size-grading. The results from *Experiment 8.2* indicate that it will not be sufficient to select for reduced aggression in culture, but that genetic variation may have to be reduced if selective breeding is to be effective in controlling growth variation.

8.5 SUMMARY

1. The physical properties of tubifex significantly affected growth, which was highest in the diets which most closely resembled natural food, and was probably related to the bioenergetic costs of location, capture and ingestion.

2. Social interaction was reduced to a few minutes at feeding times in gravel tanks, where growth rate was near optimum and mortality prevented, although there was no change in growth variation.

3. It appears that growth variation has some genetic origin and reduction of the phenomenon in culture may require selective breeding.

CHAPTER 9

THE EFFECT OF STRESS IN CULTURE

9.1 INTRODUCTION

The unequal growth of size-matched fish in culture is believed to be determined by individual genetic differences in aggression, sex or prior social experience (Knights, 1985), or by differences in vigour, or susceptibility to stress, which develop as a result of some 'genetic predisposition' (Kuhlmann, 1974).

The stress response is an adaptive mechanism, or series of adaptive responses, with which an animal attempts to regain homeostasis when faced with an external stress or stimulus (Pickering, 1981). Components of this response have been collectively known as the General Adaptation Syndrome (GAS) which were divided into stages of alarm, resistance and exhaustion (Seyle, 1956). A series of stress responses are induced by a stimulus or 'stressor'. In teleostean fishes, the primary response to stress involves neural and hormonal stimulation which elevate the levels of catecholamines and corticosteroid hormones. This affects energy mobilization and hydromineral balance (Schreck, 1981), leading to secondary changes in behaviour, physiology and morphology (Mazeaud *et al.*, 1977). Tertiary changes may be induced, such as reduced appetite and increased susceptibility to further stress or disease (Wedemeyer and McLeay, 1981). Under natural conditions this might involve flight but in intensive culture, where escape is not possible, stress can be a physiological burden (Peters *et al.*, 1980).

Any factor which causes fright, discomfort or pain will induce a stress response, where perfect compensation can be achieved by the GAS even if the stress is chronic (Schreck and Lorz, 1978). However, performance capacity of the fish is reduced during the time between alarm and compensation, due to the costs associated with regaining homeostasis. Recovery is dependent upon the magnitude and duration of the stress, and may include hyperphagia, so that long term growth is little affected (Pickering *et al.*, 1982).

The stress response can be measured by quantifying the immediate physiological responses and many of the neuroendocrine and physiological responses of fish to stress have been reviewed in individual papers by Donaldson, Eddy, Hughes, Mazeaud and Mazeaud (1981). Another approach is to measure long-term changes in performance capacity of the fish in terms of growth rate, reproductive

success and ultimately, survival. This could take the form of specific challenge tests which are useful for determining tolerance limits to stresses, including social interaction (Knights, 1985).

Stressors associated with intensive fish culture induce both acute and chronic stress responses. Acute stress can result from handling procedures such as grading or transportation or, in experimental culture, due to handling associated with growth assessment. The effects of secondary changes in osmotic and ionic regulation can be reduced by using dilute saline solutions when handling or transporting fish in freshwater (Eddy, 1981). Chronic stress can result from deteriorating water quality (Smart, 1981) and social interaction (Schreck, 1981).

Ammonia in freshwater, in its un-ionized form (NH_3), is growth limiting at sub-lethal levels, that is, above 0.12 mg. l^{-1} (Sadler, 1981) and can be a problem in recirculation systems if allowed to accumulate where ammonia is produced as the end product of protein metabolism. Ammonia toxicity increases with high pH, low dissolved oxygen and increased temperature. This is most likely to occur after feeding and especially with high protein diets, where the demand for dissolved oxygen increases, known as specific dynamic action. Temperature induced stress was demonstrated in *Anguilla anguilla* where the delay in return to full appetite was proportional to the duration of exposure to a low temperature shock (Knights, 1985).

Social interaction induces stress due to aggression and competition, with submissive fish showing enhanced interrenal activity (Schreck, 1981), reduced growth rate and increased susceptibility to infection (Peters *et al.*, 1988). In *Anguilla anguilla* gastric mucosal atrophy (Peters, 1982) and enlarged gall bladders (Willemse *et al.*, 1984) have been attributed to stress. There may also be a psychological component to stress in fish populations where social status is associated with a particular state of stress, for example, in coho salmon *Oncorhynchus kisutch* subordinates demonstrated a higher level of clinical stress (Schreck, 1981).

An unsuitable diet which results in a reduction in growth rate can be considered a stressor. This could be due to different nutritional qualities (Koops and Kuhlmann, 1981) or physical properties of the diet, as shown in Chapter 7 and *Experiment 8.1.*

The additive or synergistic effects of multiple stressors on fish performance is likely in intensive culture, for example, overcrowding and water quality deterioration may provide favourable conditions for transmission and survival of pathogens, whilst stress may induce immunosuppression in the fish (Pickering, 1981).

This chapter describes two experiments which were designed to examine the effect of stress induced by type of diet and social interaction, where the stress response was measured in terms of growth rate and survival.

In ***Experiment 9.1*** a stressor and non-stressor were denoted for each factor examined, which were identified from results of previous experiments. That is, for type of diet, a dry diet stressor and live tubifex non-stressor, where growth and survival were near optimum on a live tubifex diet (Chapter 7 and *Experiment 8.1*) and for social interaction, a non-gravel stressor and gravel non-stressor, where social interaction was reduced to a few minutes at feeding time in the gravel tanks (*Experiment 8.1*). The response of the same population of fish when subjected to the stressors and non-stressors in a cross-over sequence was examined.

In ***Experiment 9.2*** the effect of hierarchical position on the stress response was examined in populations of eels which were individually marked. A measure of the dominance hierarchy was made by recording the number of bites sustained by each fish from aggressive chase and bite behaviour. Any effects of stress on body composition were examined by proximate analysis of individual fish.

9.2 METHODS (The culture system and holding, transfer, experimental and analytical methods are described in Chapter 1.2.2)

9.2.1 *Experiment 9.1*

Design

The stressors and non-stressors for type of diet and social interaction were arranged in four different treatments. Two contrasting treatments were given in a cross-over sequence to the same population of fish, over a period of nine weeks. Cross-over of the treatment occurred twice, after week 3 and week 6. Each population of fish acted as its own control and the cross-over sequences were replicated twice. The experiment was designed as shown in Figure 9.1 but was adjusted because of high mortality in treatments B and D.

Figure 9.1 Original design of *Experiment 9.1* (*= treatment sequence discontinued)

Treatments		TYPE OF DIET	
		Tubifex	Dry diet
SOCIAL INTERACTION	Gravel	A	C
	Non-gravel	B	D

Cross-over sequence

x1	x2
A B	A
B A	B
C D	C
D C	D
B D	B*
D B	D*

In the first 3 weeks survival of fish in the four replicates on treatment B was 25 %, 15 %, 15 %, and 0 % (shown as additional replicates in lower case, babl to bab4, in Figure 9.2). The 21 surviving fish from treatment B were grouped and ranked into 2 replicates of similar coefficient of variation, BAB1 and BAB2, and the treatment sequence was continued (Figure 9.2). Mortality was also high in treatment D and the two replicates with fewest survivors were discontinued, that is, where survival was 55 % and 40 % (shown as additional replicates, dcdl and dcd2, in Figure 9.2).

Fish

Elvers were obtained commercially from 'Bristol Channel Fisheries' (River Severn, UK). They were graded into 50 mg groups from 0.150 mg to 0.349 mg and the groups were randomly assigned to 12 x 10 litre tanks for treatments A, B, C and D, 20 fish per tank. There were two replicates for each treatment consisting of fish of similar size groups except for treatment C (Figure 9.2). Fish were weighed and measured after 6 days feeding and 1 days starvation, except for the first week, when the fish were undisturbed to allow them to acclimatize to the experimental conditions.

Diet

Live tubifex was fed at 20 % b.wt.day^{-1} and B.P. dry diet at 5 % b.wt.day^{-1} and the ration was adjusted after each weighing period.

Substrate

1 litre of washed gravel (Dorset pea gravel) was inserted into tanks on treatments A and C and a similar maintenance procedure was followed as described in *Experiment 8.2*.

9.2.2 *Experiment 9.2*

Marking

Fish were anaesthetized and individually marked (Riley, 1966) with a subcutaneous injection of acrilic paint (Windsor and Newton Ltd, England). It was not possible to mark fish less than 1g in weight without damaging them. The needle was inserted at 5°, anterior to the dorsal fin to avoid impairment of tail musculature, and precautions were taken to avoid infection by sterilizing the needle in 75 % ethanol between injections (Seymour, 1984). The marking needed to be renewed for a few fish during the experiment.

Fish

Elvers were obtained from 'Bristol Channel Fisheries' and kept in holding facilities until they reached 1g. They were marked and returned to another holding tank, to allow them to settle, during which time a few developed wounds around the acrylic mark and were removed. Fish were graded to include fish between 1 to 5 g which were randomly assigned to 12 x 10 l tanks, 5 fish per tank. Weight and length were recorded every 12 days, after 1 days starvation, and the regime was continued for 72 days. Dead or moribund fish were removed and their weight and length recorded.

Diet

The experiment was originally designed to test different levels of protein nutrition which consisted of two replicates on each protein level as follows:

Diet		**Replicates**
casein-based	60%	1.1, 1.2
fish meal-based	30%	2.1, 2.2
	40%	3.1, 3.2
	45%	4.1, 4.2
	50%	5.1, 5.2
	60%	6.1, 6.2

For the purposes of this chapter the effects of differences in nutrition are borne in mind but are incidental to the objective of looking at growth in relation to social interaction. The diet was dispensed twice a day at a feeding rate of 6.5% body weight day^{-1} in paste form. Dry diet was made into a paste with addition of 10 % water at each feeding time. A paste diet was used, in preference to pellet or crumb, to avoid the effect that particle size may have on food allocation in a population of fish of mixed size.

Body composition

Proximate analysis of individual fish was performed at day 72 or on moribund fish that were removed during the experiment (Chapter 5.2).

Aggression index

The occurrence of bite marks, resulting from aggressive encounters with other eels, was recorded during anaesthesia on a scale of 1 to 5, that is, 1 = single bite; 2 = two bites; 3 = more than two bites; 4 = bleeding and 5 = caudal vertebrae visible.

9.3 RESULTS

9.3.1 *Experiment 9.1*

Changes in median weight, coefficient of variation, instantaneous growth rate and survival over the trial period are shown in Figure 9.2. The correspondence of replicates was high in all treatments. The population median weight increased in all treatments over the experimental period and this trend was unaffected by cross-over, except in BAB1 and BAB2, where there was a decline in median weight after cross-over 2, which was matched by a sharp reduction in growth.

Coefficient of variation increased in all treatments over the trial period. A decrease from one week to the next coincided with mortality (CDC1, DCD1 and DCD2) or decreased growth rate (BAB1 and BAB2). Coefficient of variation was highest in treatment ABA1 and ABA2, that is, 50.9 % and 48.7 % respectively.

Mortality was largely due to aggressive chase and bite behaviour. The dead fish had bite marks on their tails and were generally the smaller individuals. Survival was affected by treatment and was highest in the gravel, that is, 85 % and 100 % on tubifex and 90 % and 100 % on dry diet, during the first 3 weeks. Survival was much lower in the non-gravel during this time, that is, 25 %, 15 %, 15 % and 0 % on tubifex, and 90 %, 75 %, 55 % and 40 % on dry diet. At cross-over 1, mortality increased in the treatments changing from gravel to non-gravel, that is, ABA2, CDC1 and CDC2, and continued in tanks changing from non-gravel to gravel, that is, BAB2, DCD1 and

DCD2. At cross-over 2, survival was high on the tubifex diet in gravel and non-gravel, whilst mortality continued on the dry diet in the gravel and non-gravel, that is, CDC1 and DCD2.

Instantaneous growth rate was highest on a tubifex diet in the gravel and on a dry diet in the non-gravel. After cross-over from non-gravel to gravel, growth rate increased on the tubifex diet and decreased on the dry diet. However, the initial drop in growth rate at cross-over did improve in both dry diet in gravel and tubifex in non-gravel.

FIGURE 9.2 *(Experiment 9.1)* Change in median weight, coefficient of variation, instantaneous growth rate and % survival: Appendix 9.1

(A=tubifex+gravel, B=tubifex+non-gravel)

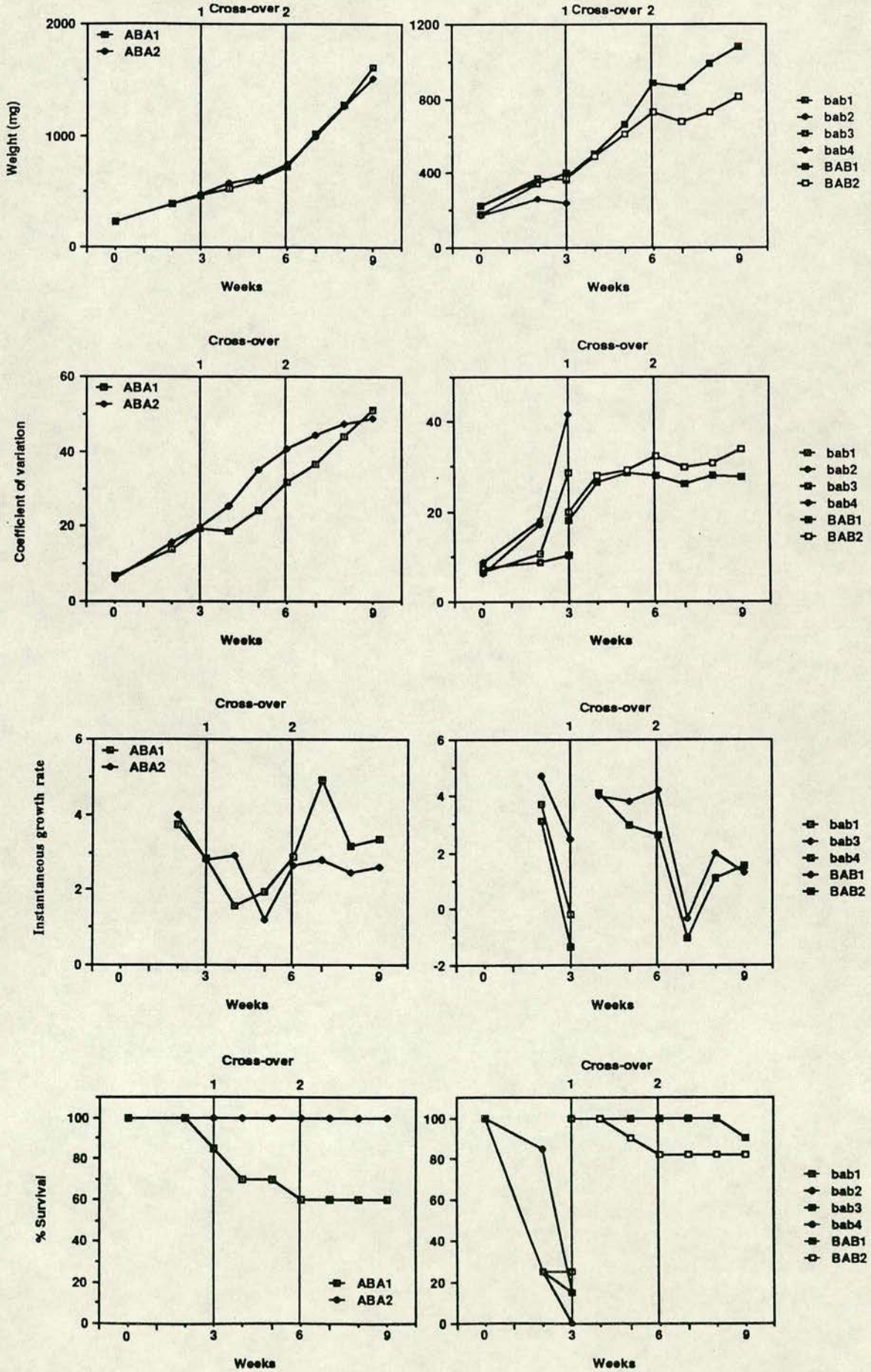
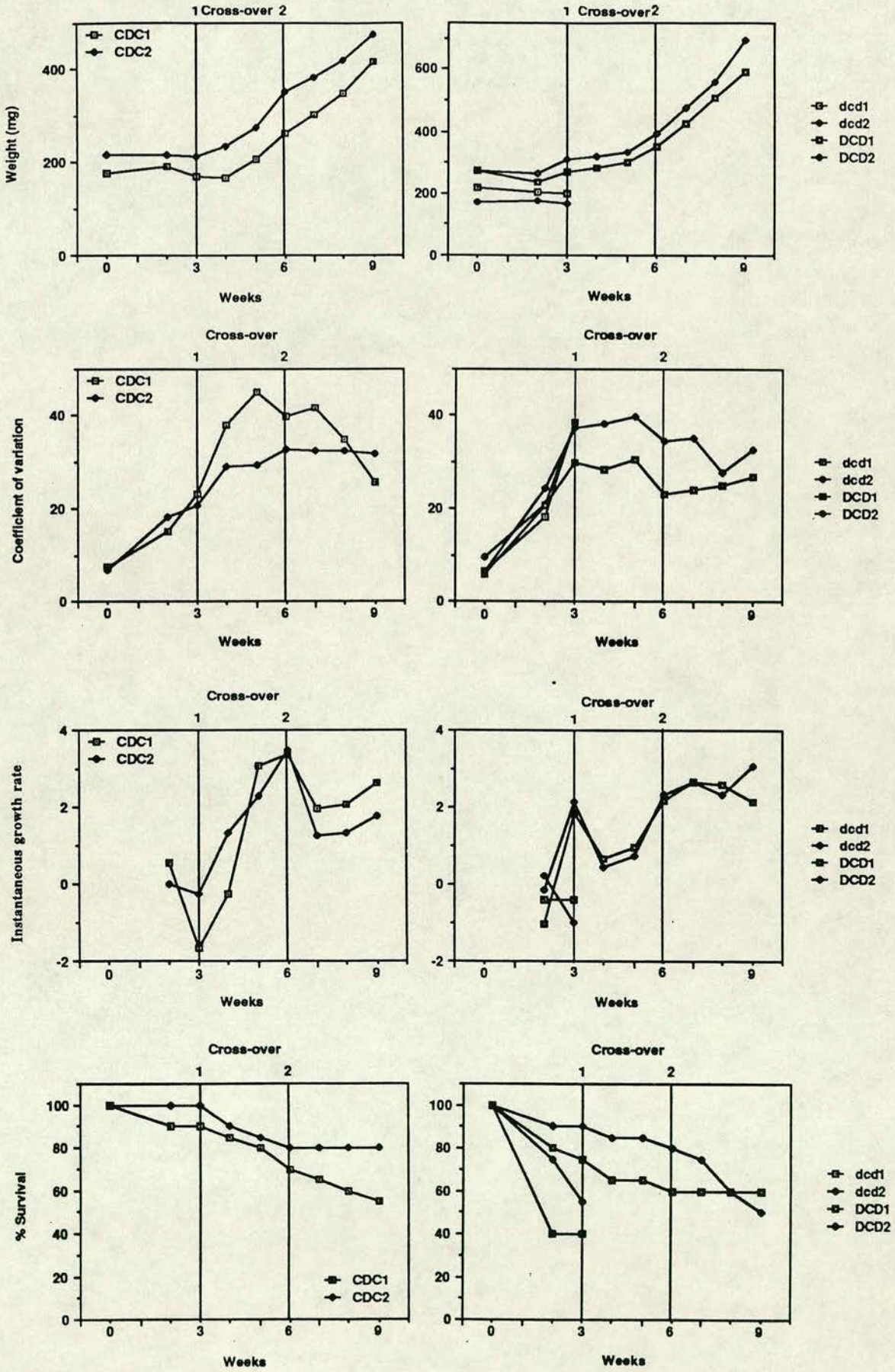


FIGURE 9.2 continued. *(Experiment 9.1)* Change in median weight, coefficient of variation, instantaneous growth rate and % survival: Appendix 9.1 (C=dry diet+gravel, D=dry diet+non-gravel)



9.3.2 *Experiment 9.2*

The changes in weight and length of individual fish over the experimental period and the body composition of each fish are shown in Figure 9.3. The amount of aggression each fish was subjected to is indicated, and escaped fish, and dead or moribund fish, that were removed, are marked, 'e', or remain unmarked respectively.

Growth varied between replicates on the same diet, for example, tanks 2.1 and 2.2, which indicated that factors other than nutrition were involved in determining the pattern of population growth. Instantaneous growth rate (G) was determined at day 72 or, due to the high mortality, when there were three fish remaining in the tank (day 't'). Because of the small number of fish in each population, coefficient of variation was not appropriate as a measure of growth variation, therefore, the divergence (D) between the largest and smallest fish in each tank was determined. D was expressed as a percentage (largest / smallest x 100) at the start of the experiment and when aggression was first recorded (day 't') or, on day 72 if there was no aggression recorded. The total score on the aggression index (A) and mortality (M) for each population are also shown in Table 9.1.

Most fish maintained their original position in the size hierarchy in each tank population. In some populations the size hierarchy persisted throughout the experimental period for both length and weight (tanks 2.1, 4.2 and 5.1) and in others there were slight overlaps with weight hierarchies (tanks 2.2 and 5.2). In the other tanks readjustment of hierarchical position was quite common during the first 12 day period, after which the positions were maintained (tanks 1.2, 3.1, 4.1 and 6.2). Occasionally, rapidly growing fish developed from lower down the original size hierarchy (tank 3.1, fishes 2 and 4 and tank 1.2, fish 1) although it was more usual for the smaller fish to maintain or lose weight, becoming the 'runt' in some populations (tank 1.1, fish 2; tank 3.2, fish 4 and tank 6.1, fish 4).

Divergence in size was high at the start of the experiment in some populations, that is, D% for weight ranged from 17 % to 254 %, in tanks 3.2 and 5.2 respectively, and for length, ranged from 4 % to 40 %, in tanks 1.1 and 4.2 respectively. Divergence in size increased by day 72 or day 't' where the range for weight was 73 % to 362 %, in tanks 1.1 and 5.2 respectively, and for length was 6 % to 58 %, in tanks 1.1 and 4.2 respectively.

FIGURE 9.3 (*Experiment 9.2*) Change in weight (W) and length (L) and proximate analysis of individual fish : Appendix 9.2

(e=escaped, 1-5=aggression index)

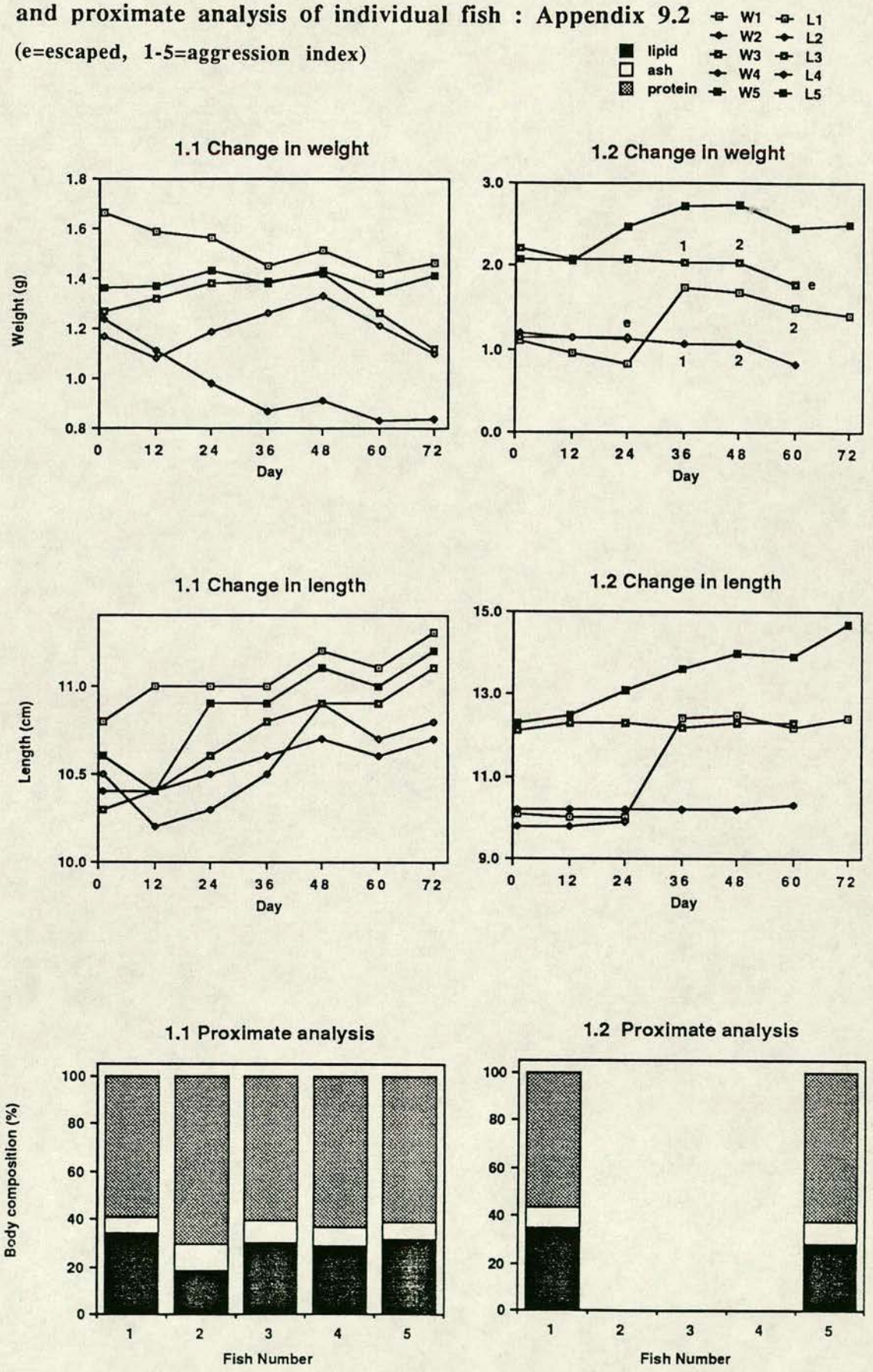


FIGURE 9.3 continued. (*Experiment 9.2*) **Change in weight (W) and length (L) and proximate analysis of individual fish : Appendix 9.2**

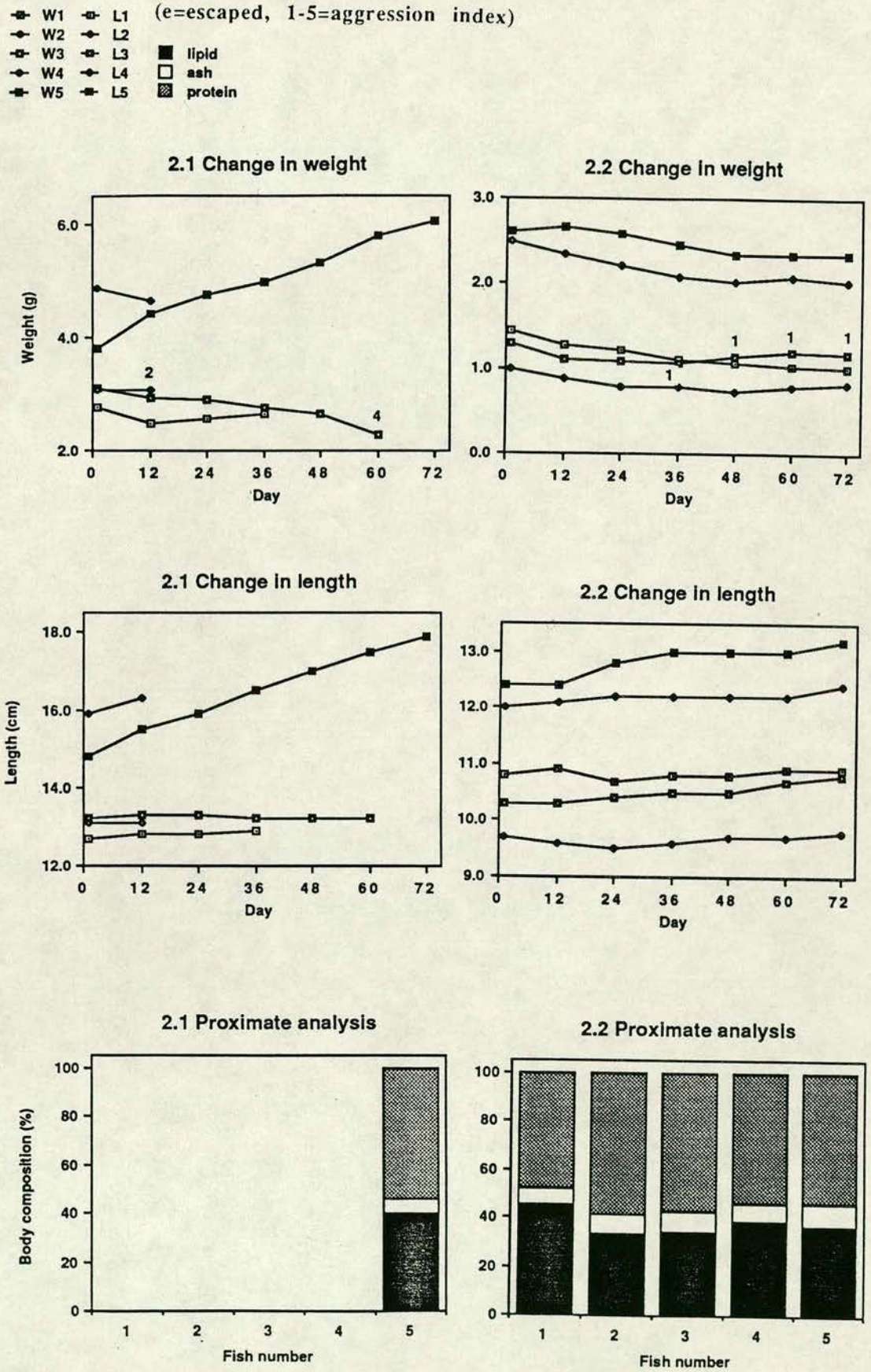


FIGURE 9.3 continued. (*Experiment 9.2*) Change in weight (W) and length (L) and proximate analysis of individual fish : Appendix 9.2

W1 L1 (e=escaped, 1-5=aggression index)
W2 L2
W3 L3 lipid
W4 L4 ash
W5 L5 protein

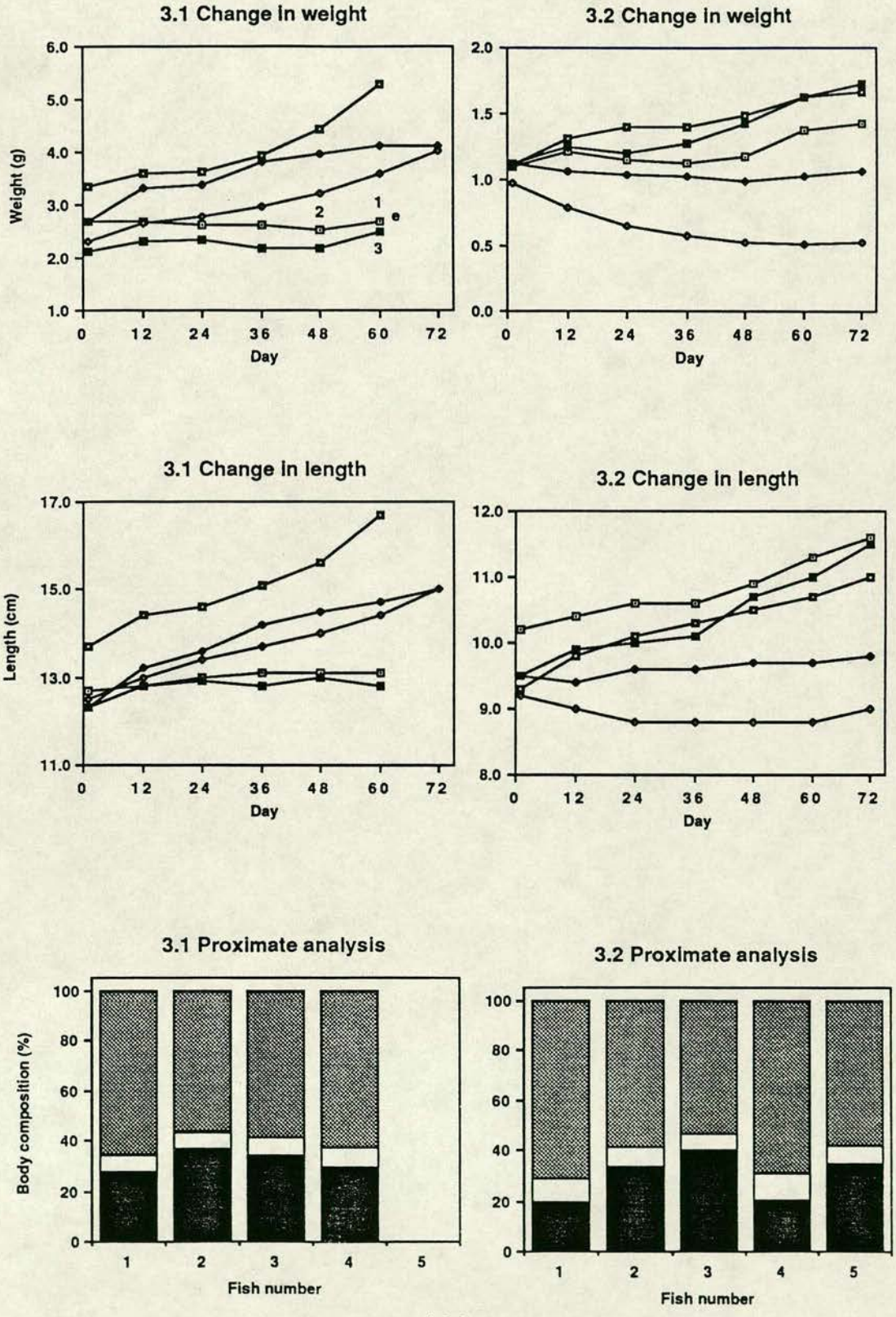


FIGURE 9.3 continued. (*Experiment 9.2*) Change in weight (W) and length (L) and proximate analysis of individual fish : Appendix 9.2

W1 W2 W3 W4 W5 L1 L2 L3 L4 L5 (e=escaped, 1-5=aggression index)

lipid ash protein

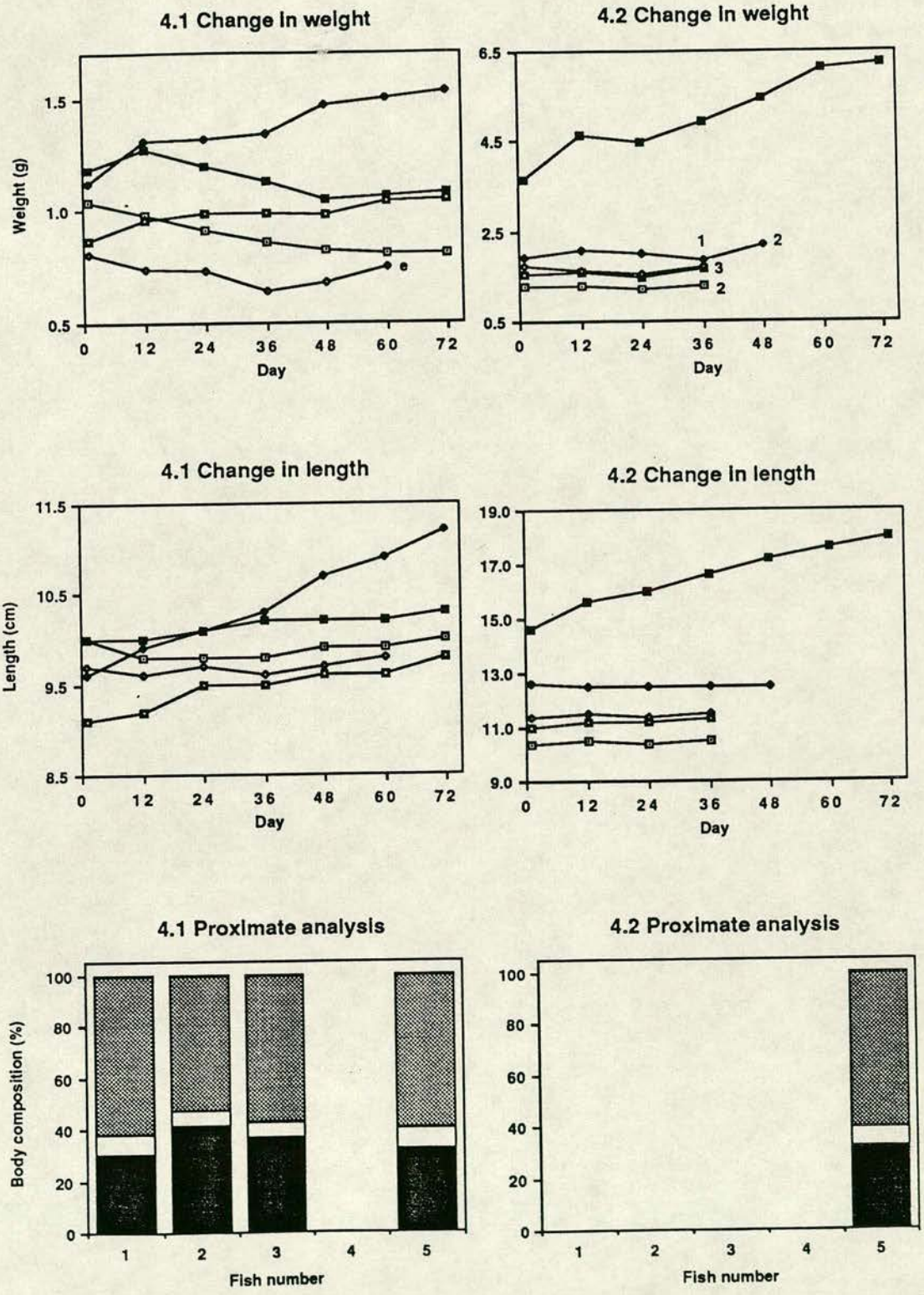


FIGURE 9.3 continued. (*Experiment 9.2*) Change in weight (W) and length (L) and proximate analysis of individual fish : Appendix 9.2

W1 W2 W3 W4 W5 L1 L2 L3 L4 L5 (e=escaped, 1-5=aggression index)

lipid ash protein

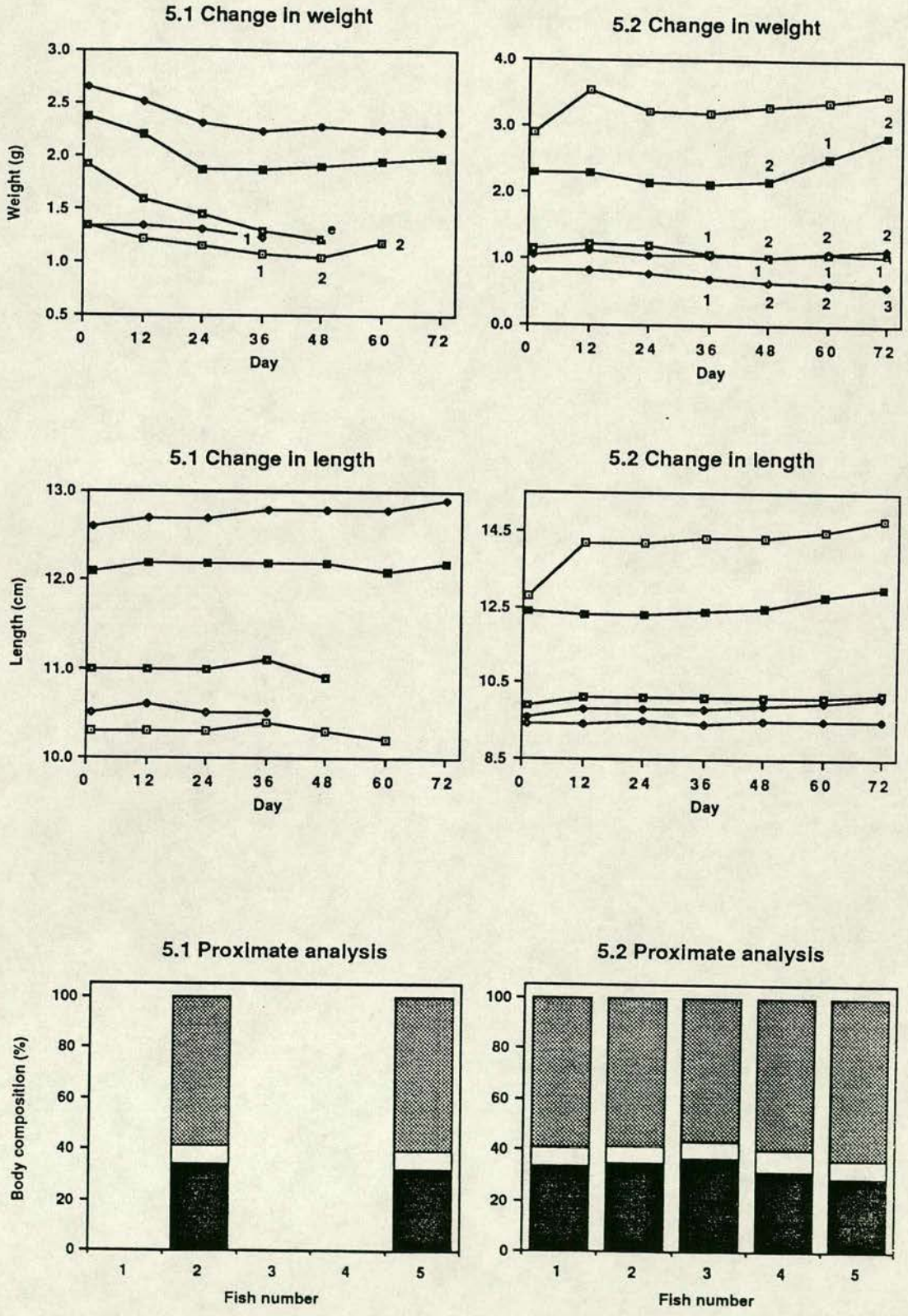


FIGURE 9.3 continued. (*Experiment 9.2*) Change in weight (W) and length (L) and proximate analysis of individual fish : Appendix 9.2

W1 W2 W3 W4 W5 L1 L2 L3 L4 L5 (e=escaped, 1-5=aggression index)

lipid ash protein

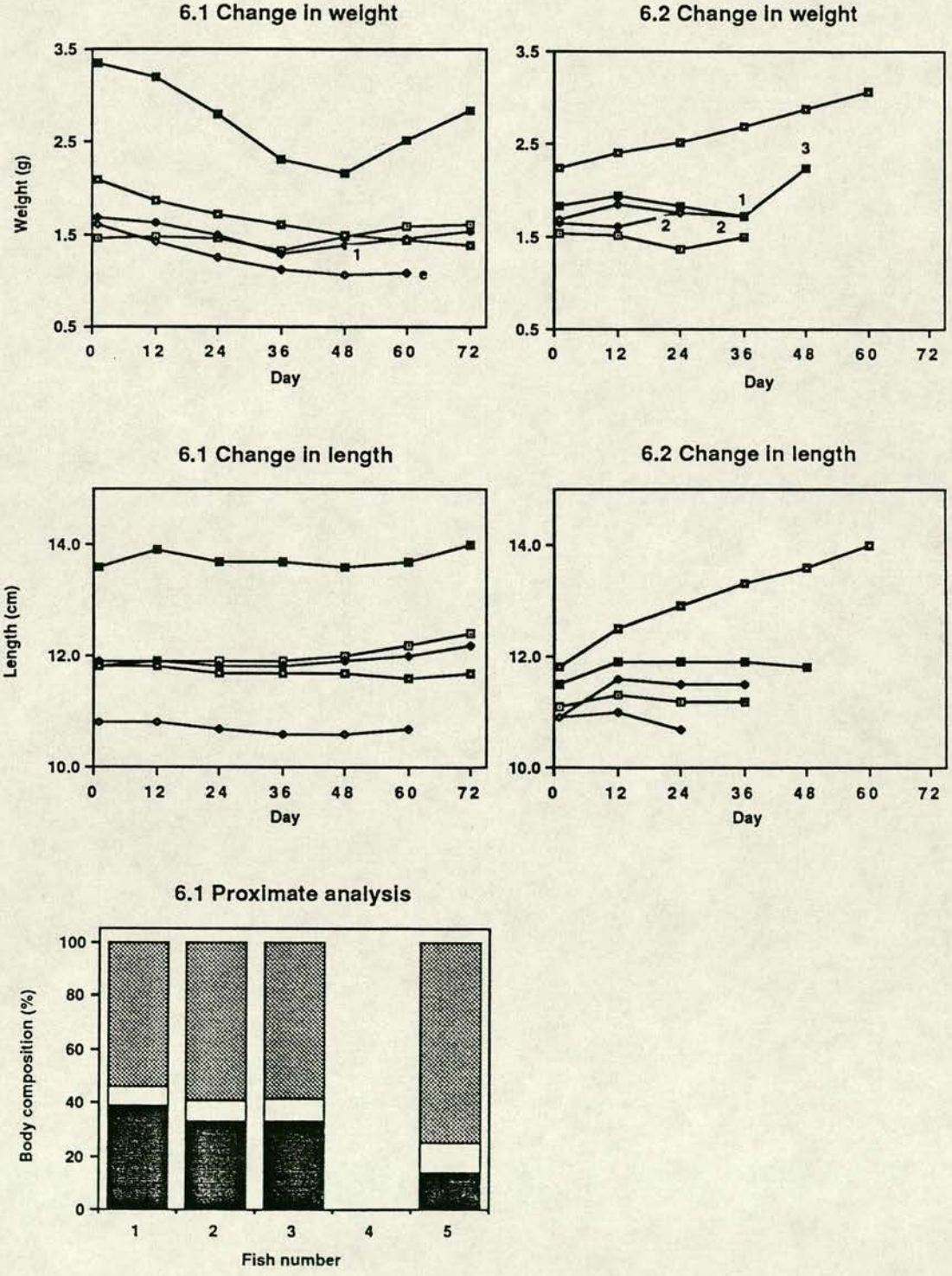


Table 9.1 ***(Experiment 9.2)*** **Instantaneous growth rate (G), divergence (D%), aggression (A) and mortality (M) : Appendix 9.2** (w=weight, l=length, i=initial, f=final,)

	G			D%				A	M
				w		l			
Tank	Day	w	l	Day	i	f	i	f		
1.1	72	-0.18	0.08	72	42	73	5	6	0	0
1.2	60	0.53	0.31	36	99	156	26	33	6	1
2.1	36	-0.32	0.00	12	76	86	25	27	6	4
2.2	72	-0.28	0.00	36	161	187	28	35	2	0
3.1	60	0.49	0.24	48	57	103	11	20	5	2
3.2	72	0.35	0.20	60	17	219	11	28	1	0
4.1	72	0.00	0.07	72	46	89	9	14	0	0
4.2	36	-0.06	0.00	36	186	284	40	58	7	4
5.1	60	0.03	0.16	36	98	107	22	23	3	2
5.2	72	0.01	0.04	36	254	362	36	52	8	0
6.1	72	-0.09	0.06	48	131	103	26	28	1	0
6.2	36	0.03	0.15	24	47	85	8	21	7	5

Growth was not high in any of the populations. G was highest in tank 1.2, that is, 0.53 % body weight per day and lowest in tank 2.2, that is, -0.28 % day^{-1}. There was no decrease in length, but there was virtually no growth in length in tanks 2.1, 2.2 and 4.2, and growth in length was highest in tank 1.2, that is 0.31 % day^{-1} Individual G in weight ranged from -0.54 % to 0.77 % day^{-1} in tank 1.1, fish 2 and tank 3.1, fishes 3 and 4 respectively. Length measurements were accurate to $\pm$ 0.1 cm and only two fish decreased in length below this, that is, by 0.3 cm and 0.2 cm in tank 1.1, fishes 4 and 5 respectively.

The levels of the different body composition constituents varied considerably between fish in some tanks and was not, therefore, wholly due to level of protein nutrition (Appendix 9.2). Ash levels ranged from 5.85 % to 11.54 %, protein level ranged from 48.27 % to 75.30 % and the range in lipid level extended from 13.77 % to 45.16 %. The lipid level of most fish was between 30 to 40 % and was very even between fish in some populations and extremely variable in others, for example, tanks 2.2 and 5.2 and tanks 3.2 and 6.1 respectively. In some populations the largest fish

had a high lipid level and low ash level, for example, tank 2.2, fish 1, lipid 40.11 % and ash 6.57 % and tank 4.1, fish 2, lipid 41.14 % and ash 6.02 %, although the largest fish in tank 6.1, fish 5, had the lowest lipid level, that is, 13.77 % and high ash level, that is, 10.93 %. The two runts which survived to day 72, had low lipid levels and high ash levels, that is, tank 1.1, fish 2, lipid 18.08 % and ash 11.54 % and tank 3.2, fish 4, lipid 20.28 % and ash 10.72 %.

The interrelation of size and dominance hierarchies was demonstrated by the aggression index, which was zero in the largest or dominant fish and highest in the smaller subordinates. Social interaction was high and the degree of aggression recorded from chase and bite behaviour was dependent upon the divergence in size of individual fish in the population. There were no bites recorded until a length differential between 15 % to 20 % developed between the largest and smallest fish, for example, there were no bites on fish in tanks 1.1 and 4.1, where the length divergence was 6 % and 14 % respectively at day 72. There were no bite marks on dominant fish, although they were found on many of the subordinates, which often lead to mortality, possibly through osmotic imbalance. This could be due to a simple morphometric relationship, where mouthsize is proportional to length, with smaller fish being unable to hold the tail of a larger fish in their mouth (Knights, 1982). One dominant fish died at day 60, that is, tank 6.2, fish 3, which may have been due to the demands of hierarchical defence.

The degree of aggression recorded in each population varied from 0 to 8, in tanks 1.1 and 4.1 and tank 5.2 respectively. The highest score for an individual fish was 4, that is, caudal fin bleeding in tank 2.1, fish 3. Mortality varied from 0 %, in tanks 1.1, 2.2, 3.2, 4.1, 5.2 and 6.1 to 100 %, in tank 6.2. Two fish escaped from tank 1.2 and one fish escaped from tanks 3.1, 4.1, 5.1 and 6.1.

9.4 DISCUSSION

9.4.1 *Experiment 9.1*

The response to the treatments was dependent upon the combination of diet and social interaction, and to a lesser extent, to the sequence of the treatment in the cross-over. In some treatments, the parameter used to measure the stress response, that is, survival or growth rate, determined whether type of diet or absence of gravel was considered a stressor or non-stressor.

Highest growth rate and survival was obtained with live tubifex and gravel, the suitability of which have been discussed previously in *Experiments 8.1* and *8.2*. Social interaction was reduced to a few minutes at feeding time with a gravel substrate, and consequently, survival was high and energy expenditure low, with rapid growth on the highly suitable, live tubifex diet. Growth variation was high. Social interaction and mortality were greatly increased without the gravel substrate, and mortality was highest on the tubifex diet, where growth rate was most rapid. Growth variation is not prevented by less social interaction but the rate of development of the size differential is reduced. The rate of development of a size differential increases with growth, and the damaging effects of chase and bite behaviour increase, as seen in *Experiment 9.2*. Rapidly growing fish may also have greater energy resources and show higher aggression. Survival was enhanced on the dry diet in gravel, but growth rate was low compared with non-gravel, despite the reduced social interaction. This was probably because the fish could not pick-up the food easily from the gravel, which lead to wastage of part of the ration.

The sequence of the treatment in the cross-over affected the stress response. Social interaction in the non-gravel resulted in high mortality during the first three week period, and least mortality after cross-over 2, where the fish were becoming acclimatized to the new conditions (Kuhlmann, 1974). The frequency of agonistic encounters is highest when fish first interact, as linear dominance hierarchies are established (Knights, 1985). Also, the earlier loss of weak fish may improve later survival in the population. The effect of changing treatments on growth rate was clearly shown at cross-overs 1 and 2, where the response of both replicates corresponded well and the contrasting treatments mirrored each other. The capacity to reverse the stress response after a duration of three weeks, and the capability to do so for a second time, was demonstrated.

9.4.2 *Experiment 9.2*

The relative position of an individual fish in the size hierarchy of the population remained fairly constant throughout the experimental period, whether the population gained or lost weight. There was slightly more overlap between individuals in the weight hierarchy. Fish that did not adopt a position in the size hierarchy, relative to their initial size, may have been reacting from prior social experience (McDonald *et al*, 1968) or due to genetic differences, which gave them a greater or lesser advantage in the new population. The pattern of population growth was not dependent upon the initial size distributions as the highest population growth resulted from a wide range of initial size distributions, for example, where divergence was 17 %, 57 % and 99 %. Also, fast growing fish or 'shooters' developed from a wide range of initial size distributions, for example, where divergence was 17 %, 47 % and 186 %.

The effect of acclimation to the experimental conditions was evident during the first 12 day period where there was most readjustment of hierarchical position.

In populations that were growing, social interaction reinforced the hierarchy and increased the size divergence, which lead to the smaller fish losing weight. Loss of weight was probably due to the energy expenditure involved with avoiding dominant fish and being forced to stay in the water column (Knights, 1985; Wickins, 1987) and that thay were not obtaining sufficient food. A reduced food intake has been found in situations where the diet was provided in excess (Purdom, 1974) and suggests that appetite is suppressed by social interaction. The diet was presented in paste form, to avoid the effect that pellet or crumb size may have had on ration allocation, in a population of fish of mixed size, however, this may not have been suitable because the high social interaction broke the paste into a particulate form, and there was some wastage of the ration.

There were no bite marks resulting from chase and bite behaviour until there was a length differential of 15 to 20% between the smallest and largest fish in a population, whereas, the effect of differences in weight were not as clear. However, dominance is reported to be strong with differences in weight of 150%, or when a fish is 1.5 times larger than another (Knights, 1985).

The body composition of the runts and dominant fish, which had low and high levels of lipid repectively, was of a similar pattern to the slow and fast growing immature eels in the wild (see Chapter 5), although lipid levels of the experimental fish were generally higher. However, one dominant fish had a low lipid level, which may have been due to lipid depletion, where the energy requirement for defence of the hierarchy was not provided by food intake. Dominant rainbow trout *Salmo gairdneri* have also appeared to be stressed (Noakes and Leatherland, 1977). The range in body

composition constituents was slightly less in the experimental fish, compared with immature wild eels from the River Almond, which was probably due to the differences in body size and the environmental conditions of the respective populations (Table 9.2).

Table 9.2 Comparison of the range in body composition of experimental eels and wild immature eels from the River Almond

	Wild		Experimental	
	mini -mum	maxi -mum	mini -mum	maxi -mum
weight (g)	1.18	81.02	0.52	6.24
length (cm)	10.0	37.5	9.0	18.0
% dry weight				
lipid	6.72	53.94	13.77	45.16
protein	41.39	83.29	48.27	75.30
ash	3.99	17.19	5.85	11.54

Stressful conditions were imposed in both experiments where compensation was not made and performance capacity of the fish was reduced. Environmental conditions reduce the genotypic performance capacity and stress reduces performance further (Schreck, 1981). Conversely, the performance realized by some fish exceeded that expected from the environmental conditions such as relative position in the size hierarchy (Wickins, 1987), which suggests that genotypic performance capacity is variable or that prior experience influenced performance.

Social interaction had a particularly stressful, and often lethal, effect in both experiments which was probably exaggerated compared with commercial culture, where dominance hierarchies can be reduced by high stocking densities, for example, 25 kg. m^{-3} (Seymour, 1984) although subordinates are less able to escape (Knights, 1985). Periodic size-grading, to maintain homogeneous populations, breaks down size hierarchies, which develop again in the new population (Wickins, 1983). Growth was highest where divergence was high, and population growth may rely, to some extent, on the development of dominant fish at the expense of subordinates.

9.5 SUMMARY

1. Social interaction was a stressor, causing a reduction in growth rate and survival. The rate of development of a size differential was increased by social interaction, but growth variation was not prevented when social interaction was minimal.

2. Mortality was most severe when there was high social interaction of rapidly growing fish. This was related to development of a length differential of 15 to 20 % between the smallest and largest fish in a population, which allowed the larger fish to bite the smaller individuals.

3. The reversibility of a stress response was demonstrated.

4. Hierarchical position was determined largely by relative size of an individual in the newly formed population although it may have been influenced by prior social experience or genetic factors.

5. There were similar differences in body composition between slow and fast growing eels in culture and in the wild.

6. The genotypic performance capacity is reduced by the environmental and stressful conditions in culture.

7. Population growth may rely, to some extent, on the development of dominant fish at the expense of subordinates.

CHAPTER 10

DISCUSSION

There is size variation apparent at the glass eel stage of *Anguilla anguilla*. Glass eels are semi-pelagic during the migration and there is little or no feeding (Tesch, 1973) and probably little aggression as shoaling fish tend to be less excitable (Blaxter and Holliday, 1963). Until feeding commences, variation in size probably results from genetic factors and development during the leptocephalus larval stage and shrinkage during pigmentation (Schmidt, 1906; 1912). Geographical origin and time of capture account for some variation between samples (Table 10.1).

TABLE 10.1 Size of glass eels from different geographical locations

Length (cm)	Source	
6.7 - 8.1	River Ffraw, Wales	(Sinha and Jones, 1967)
5.5 - 7.7	River Severn, England	(Strubberg, 1923)
5.4 - 7.4	Adriatic Sea	(D'Ancona, 1958)
7.2 - 7.9	River Ems, Germany	(Kuhlmann, 1974)
6.0 - 6.7	Tyrrhenian Sea	(Kuhlmann, 1974)
6.6 - 7.4	River Almond, Scotland	(Present study)

Development is delayed until glass eels join the benthos and commence feeding, from which time growth variation is apparent. The glass eel migration was delayed for 2 to 3 months in the River Almond, at Cramond weir. There was no feeding during this time and consequently no change in body composition and little development of pigmentation (Chapter 6). On changing to a more benthic life the eel becomes more solitary and territorial. Agonistic behaviour, involving competition for environmental resources, especially food and space (Wilson, 1975) will account for some growth variation. In the wild, dominance can develop into a social hierarchy which reduces the costs of competition for individuals in further competitive situations. Subordinates learn to avoid confrontation by adopting a position in the size hierarchy and rely on

threat display or avoidance swimming to maintain it. There may be some resource partitioning between different size groups of eel with preferred habitats (Moriarty 1972a; Sloane, 1984) or temporally, where smaller eels may feed at a different time than the larger fish, which are more nocturnal. Glass eels and elvers were more active during the day than the larger eels in the River Almond (Chapter 6). There was some variation in the diet composition of different sized eels in the River Almond which was related to mouth size and differences in feeding behaviour (Chapter 2). Glass eels and elvers were restricted to 10 species of food organism and glass eels showed a tendency for a more pelagic diet, that is, *Cyclops* and chironomid adults. Eels in intermediate size groups, that is, between 20 to 30 cm had the greatest species diversity in the diet, that is, more than 30 species, and showed a tendency to gather rather than pick food items. This was corroborated by the increased proportion of detritus in the stomach contents. Larger eels had a smaller proportion of oligochaetes than smaller size groups but more of the larger organisms such as *Asellus aquaticus* and *Limnaea pereger*. Larger eels, that is, over 50 cm, have been found in other studies to be more piscivorous (Frost, 1945; Moriarty, 1975).

Differences in sex may have an influence on competitive behaviour and growth variation. Most of the sexually differentiated eels in the River Almond were female, that is, 17 % of the eels sampled, whereas males made up only 1 %. Males reach maturity and begin their migration to the spawning grounds at an earlier age than the females (Aprahamian, 1988) and although smaller, are not necessarily slower growing (Cripps, 1983). The growth of the females was faster than immature eels of the same age in the River Almond (Chapter 3). The sex determination mechanism is not fully understood and the literature is often confusing and contradictory (Beeckman and Ollevier, 1987). In culture experiments, the immature, more stable stages are studied (Brett, 1979), which is also the stage which give the most problems in eel culture (Liewes, 1978). For these reasons young eels under 10 g were studied in the present growth experiments.

Other factors may affect growth, for example, diet quality and quantity, temperature, light and oxygen. In the River Almond it was estimated that conditions were suitable for growth for 19 weeks between June 1985 to June 1986 (Chapter 4), where water temperature was the major limiting factor, with increased activity and feeding above 10 °C (Chapter 2). Annual growth increments were similar to those found in other populations in temperate waters (Frost, 1945; Sinha and Jones, 1967a) (Chapter 3). Water temperature, light and dissolved oxygen were kept constant in the growth experiments (Chapter 1).

Growth variation is particularly high in *Anguilla anguilla* and the genetic

component is considered to be strong (Kuhlmann, 1974) and related to its life cycle. The fact that a large population is contributing to a single genepool increases the degree of polymorphism (Kimura and Ohta, 1971) which is maintained by the advantage this conveys in a temporally and spacially heterogeneous habitat (Somero and Soulé, 1974) and the differential survival of genotypes under different environmental conditions (Koehn, 1970). *Anguilla anguilla* is an indigenous warm water species (Sinha and Jones, 1967a) and is near the extreme of its geographical range in Scotland, where it shows greater variation and slower growth than the same species in the Mediterranean (Kuhlmann, 1974). There is probably a selective advantage in possessing a high degree of variation in the population, and of growth rate in particular, where environmental conditions are variable and often sub-optimal.

Growth rate affected body composition of eels in the River Almond (Chapter 5). Lipid level is an indicator of nutritional condition (Love, 1980). Female eels had the highest proportion of lipid which was related to their faster growth rate and high weight to length ratio. Lower nutritional condition was demonstrated in the lower lipid levels of both slow growing eels, which had lower lipid levels than rapidly growing individuals, and eels with higher length to weight ratios. If nutritional condition can be taken as an indication of general condition, the slower growing fish will have less chance of reaching maturity before they die (Alexander, 1982). A similar relationship was found between slow and rapidly growing eels in *Experiment 9.2* and was probably caused, in part, by physiological differences induced by environmental factors such as competitive stress.

Growth variation is more extreme in culture than in the wild and is probably related to the increased water temperature and access to food, and to agonistic behaviour which causes growth depensation, where larger fish grow at a faster rate than the smaller individuals which lag further behind (Purdom, 1974). In *Experiment 9.2* the interrelation between the dominance and size hierarchies was shown with an aggression index, which was zero in the larger dominant fish and highest in the smaller subordinates. It is likely that a social hierarchy is prevented from developing by culture conditions and, therefore, a dominance hierarchy will persist. Subordinates cannot escape in culture tanks and threat displays or feature recognition may be impossible at high stocking densities, although learning of hierarchical position may help in avoiding confrontation. Larger fish continue to be aggressive after they become dominant, they may learn dominance, which would be an advantage to maintain in the wild in holding territorial resources. Aggressiveness could also be genetic, inheritance of aggressiveness has been reported in the convict fish *Cichlasoma nigrafasciatum* (Schroder, 1975 in Browne, 1981). If it is genetic, then

aggressiveness may continue until avoidance by subordinates, which may not be possible under culture conditions (Knights, 1987). In some situations the dominant fish may not be the largest fish (Browne, 1981) and aggressiveness might continue, for example, the dominant fish may not secure enough food because of time taken defending territory. Social rankings may change with the resource contested, or with time, or in new populations after grading or changing conditions (Wickins, 1987).

Some of the effects causing growth variation, which have been ascribed to agonistic behaviour in eel culture are listed below, and are then followed by specific examples from other studies and the present growth experiments. Agonistic behaviour may result in growth depensation due to: social conditioning, where fish learn to adopt a position in a dominance hierarchy; disproportionate food acquisition; energy wastage, due to defending territories in dominants and avoidance action of subordinates; stress, causing loss of appetite and increased susceptibility to disease and loss of 'vigour'. The presence of water borne growth inhibitors and excreted substances has not been found in the eel and was not considered in the growth experiments.

Social conditioning may result from suppression during the holding of glass eels, even if there is no feeding at this stage, and growth depensation is seen when feeding commences, both in culture and when development is delayed in the wild. Wickins (1987) found no differences in growth of elvers graded into different size groups three days after capture, but when graded thirty days after capture, that is after becoming 'socially experienced' in the holding facilities, growth was significantly different, with smaller size groups growing less well. The growth of the different size groups of 'socially experienced' elvers remained distinct from each other. This was also seen in glass eels transferred in May, compared with June and July, all of which were socially 'naive' as regards culture, but would appear to have experienced some suppressive effect during the delay at the weir in the River Almond (Chapter 7).

Stress is associated with agonistic behaviour and is considered to be responsible for reduced appetite in subordinate eels (Seymour, 1984) causing disproportionate food acquisition in situations where food is not limiting, and thus not directly due to competition for a limited resource (Purdom, 1974). Stress also increases susceptibility to further stress or disease (Wedemeyer and McLeay, 1981). In Chapter 7 and *Experiment 8.1* survival was lower in smaller fish which indicated that mortality was due to the combined effect of aggression and infection with *Ichthyophthirius multifiliis* (whitespot). In *Experiment 9.1* and *Experiment 9.2* extreme agonistic behaviour caused mortality and reduction in growth rate. The reversibility of the stress response, where survival and growth rate increased once agonistic behaviour was

reduced, was demonstrated in *Experiment 9.1*.

Avoidance behaviour is well documented in eel culture (Knights, 1985) and was seen in all the present growth experiments, where dominant fish tended to hold preferred positions on the bottom of the tank and around the inlet and outlet pipes, whereas subordinates were forced to swim in the water column. Seymour (1984) estimated the energetic cost for subordinates to be equivalent to 0.5 % body weight day^{-1} and found that agonistic encounters and grading frequencies were reduced at stocking densities above 25 kg. m^{-3}. This was attributed to aggression being more evenly distributed throughout the population, because all fish were forced to swim at this density.

Genetic variance includes variation for growth rate *per se* or those factors which affect growth rate, including vigour in a competitive situation. Wickins (1986) isolated newly caught elvers and demonstrated that marked growth variation would occur without competition for food, or communal stresses, and that there was no relationship between growth rate and initial size. However, it was noted that the isolation of normally gregarious elvers, which were still leading a shoaling existence, may have constituted a stress, perhaps by denying communal living necessary to elicit a feeding response (Knights, 1985). In *Experiment 8.2* agonistic behaviour was greatly reduced by providing a gravel substrate in the tanks, whilst feeding remained a communal activity. The energetic costs of agonistic behaviour were greatly reduced and population growth rate was extremely high, at over 3.5 % b.wt.day^{-1} but no less variable, with a coefficient of variation similar to that in the non-gravel tanks, where agonistic behaviour was high. Survival was 100 % in gravel compared with 85 % in non-gravel where the agonistic behaviour resulted in mortality, probably induced by stress.

If genetic variation of growth rate *per se* is high in the eel, reduction of this component of growth variation would require selective breeding which is, as yet, not possible. Selection in other animals, including fish, has involved an element of domestication which has made them more suited to captivity, due in part to a reduction in agonistic behaviour. The results from *Experiment 8.2* indicate that selection solely for reduced agonistic behaviour would not be sufficient to reduce growth variation in culture, but that some reduction in genetic growth variation is required.

At present, size grading is probably the method most commonly used to minimize the effects of agonistic behaviour in eels, although the process is stressful and eels may take up to two weeks to recover and therefore, grading frequencies of no less than a month are recommended (Wickins, 1985). An alternative method, in an attempt to reduce the grading interval, is to grade when a weight differential of 1.5

between the largest and smallest fish has developed, which is associated with agonistic behaviour leading to damage from biting because the mouth of the larger fish is able to grip the tail of the smaller fish (Knights, 1982). There was not a clear relationship with weight and aggression in *Experiment 9.2*, but there was a relationship between biting and a length differential greater than 1.14 to 1.20. Grading before a weight differential of 1.5 develops would probably increase survival by reducing cannibalism and the effects of wounding, such as secondary infections and reduced osmotic balance, but it would not prevent the costs of agonistic behaviour before biting occurs, such as energy wastage and stress.

However, after grading a growth differential redevelops in the new population, which may be due to grading stress, genetic factors or prior social experience. There may be some permanency of stunting, although some stunted fish have shown remarkable recovery, that is, instantaneous growth rate over 6.5 % b.wt. day^{-1} (Wickins, 1985). In *Experiment 9.1* growth was highest where divergence was high, and population growth may rely, to some extent, on the development of dominant fish at the expense of subordinates.

Other management techniques which might reduce agonistic behaviour and its effects on growth include manipulation of stocking densities, feeding regimes, water flow velocities and provision of shelter. Low stocking densities in culture experiments, that is, below 15 $kg.m^{-3}$, may exaggerate the effects of agonistic behaviour. Maintenance of stocking densities high enough to reduce growth variation, that is , over 25 $kg.m^{-3}$ (Seymour, 1984) will present a greater disease risk and require relatively greater water flows which may not be feasible under the constraints imposed by warm water supply.

Increased water velocities may reduce agonistic encounters by directing the orientation of the fish in the current and thereby reduce the number of chance encounters between the fish, but may be difficult to maintain (Knights, 1987).

The major factors determining meal size and feeding frequency are related to the capacity and frequency of emptying of the stomach. Meals 3 to 4 times daily were found to be optimal for growth at 26.5 °C (Seymour, 1989).

Shelters have been provided to reduce agonistic behaviour in culture (Kuhlmann, 1974) but cause problems in maintaining water quality at high stocking densities. Shelter provided by the gravel in *Experiment 8.2* and *Experiment 9.1* was very effective in reducing agonistic behaviour but necessitated removal of faeces at each weighing period in the circular tanks. If a self cleaning tank could be designed to incorporate a substrate this might be worth the effort for the glass eels during first feeding, which is a critical period in culture. However, this would not be suitable for

feeding a dry diet as seen in *Experiment 9.1* but the suitability of dry food is not high as seen in *Experiment 8.1* where growth was highest on diets which most closely resembled natural food. Dry food is fed mostly in the form of paste in Japan, which is preferred to pellets because the frequency of grading is reduced where correlation between fish and pellet size is not required. In Japan the annual production in eel culture was 36,781 tons in 1979 (Gousset, 1988) compared with the U.K. where eel production was 100 tons in 1982 (Wickins, 1983). Eel production in the U.K. has declined to virtually zero by 1988, with the closure of 'Marine Farm' at Hinkley Point power station (Ingram, pers. comm.).

High density culture of *Anguilla anguilla* in temperate waters requires that the water temperature is considerably elevated. This has meant using water recirculation systems or utilizing waste heat from power stations or other industrial sources which present problems with control of water quality and lack of independence from the industry providing the source of heat.

Management techniques have been influenced by the premium on warm water which is reflected in the high stocking densities and particulate dry diets. *Anguilla anguilla* is not a natural shoaling fish, except in the non-growing migratory phases, and may not be suited to high density culture. High genetic variation is likely to be an advantage in the wild but presents enormous problems in culture, where both growth rate and factors affecting growth rate, including vigour in a competitive situation, may be genetically determined. Furthermore, *Anguilla anguilla* is not very suited to a particulate diet which exacerbates the problem of communal living.

High density culture of *Anguilla anguilla* is unlikely to be a success under conditions which impose constraints on the culture system and cause stress to the fish or until breeding in captivity and genetic selection are possible. Efficient management of the natural eel resource is not yet possible and the decline in glass eel catches during 1981 to 1986 emphasizes the need for improvement. Further knowledge of eel biology is necessary and could be used to improve the efficiency of utilization of the eel resource both through fishery and culture management.

REFERENCES

Alabaster, J.S. and Lloyd, R. 1980. Water Quality Criteria For Freshwater Fish. (F.A.O.) Butterworths, London.

Alexander, R. McN. 1982. Optima for Animals. Edward Arnold, London.

Appelbaum, S. 1980. The acceptance of granulated food by the eel (*Anguilla anguilla*, L.) and its influence on the growth of individuals. Arch. Fischereiwiss., **31**, 15-20.

Aprahamian, M.W. 1986. Eel (*Anguilla anguilla* (L.)) production in the River Severn, England. Pol. Arch. Hydrobiol., **33**, 373-89.

Aprahamian, M.W. 1988. Age structure of eel, *Anguilla anguilla* (L.), populations in the River Severn, England, and the River Dee, Wales. Aquacult. Fish. Manage., **19**, 365-376.

Association of Official Analytical Chemists (AOAC), 1975. Official methods of analysis, 12th ed. George Banta Co., Wisconsin.

Ball, J.N. 1961. On the food of the brown trout of Llyn Tegid. Proc. Zool. Soc. Lond., **137**, 599-622.

Barbour, S.E. 1984. Variation in life history, ecology and resource utilization by Arctic charr *Salvelinus alpinus* (L.) in Scotland. Ph.D Thesis, University of Edinburgh.

Beamish, R.J. and McFarlane, G.A. 1983. The forgotten requirements for age validation in fisheries biology. Trans. Am. Fish. Soc., **112**, 735-743.

Beeckman, W. and Ollevier, F. 1987. The mechanism of sex determination and differentiation in the European eel, *Anguilla anguilla*. EIFAC working party on eel, Bristol.

Bertalanffy, L. von 1957. Quantitative laws in metabolism and growth. Q. Rev. Biol., **32**, 217-231.

Bertin, L. 1956. Eels - a biological study. Cleaver-Hume Press Ltd., London.

Bieniarz, K., Epler, B., Malczewski,T. and Passakas, T. 1981. Development of European eel gonads in artificial conditions. Aquaculture, **22**, 53-66.

Blaxter, J.H.S. and Holliday, F.G.T. 1963. The behaviour and physiology of herring and other clupeids. Adv. Mar. Biol., **1**, 261-393.

Boccardy and Copper, 1963. The use of rotenone and electrofishing in surveying small streams. Trans. Am. Fish. Soc., **92**, 307-310.

Boëtius, I. and Boëtius, J. 1980. Experimental maturation of female silver eels, *Anguilla anguilla*. Estimates of fecundity and energy reserves for migration and spawning. Dana, **1**, 1-28.

Boëtius, I. and Boëtius, J. 1985. Lipid and protein content in *Anguilla anguilla* during growth and starvation. Dana, **4**, 1-17.

Boëtius, J. and Boëtius, I. 1967. Studies in the European Eel, *Anguilla anguilla* (L.). Experimental induction of the male sexual cycle, its relation to the temperature and other factors. Medd. Dan. Fisk. Havunders., **4**, 339-405.

Boëtius, J. and Harding, E.F. 1985a. List of Atlantic and Mediterranean *Anguilla* leptocephali: Danish material up to 1966. Dana, **4**, 163-249.

Boëtius, J. and Harding, E.F. 1985b. A re-examination of Johannes Schmidt's Atlantic eel investigations. Dana, **4**, 129-162.

Brett, J.R. 1979. Environmental factors and growth. In: Fish Physiology (Hoar W.S., Randall, D.J. and Brett, J.R. Eds.) Vol.VIII. Academic Press, London, pp. 599-677.

Brown, C.J. and Shipley, B.M. 1982. Soil survey of Scotland.

Browne, K.D. 1981. Aggresive behaviour and social structure in the guppy *Poecilia reticulata* a teleost. Ph.D. thesis, Polytechnic of Central London.

Bruun, A.F. 1963. The Breeding of the North Atlantic Freshwater Eel. Adv. Mar. Biol., **1**, 137-169.

Calow, P. 1985. Adaptive aspects of energy allocation. In: Fish energetics, new perspectives (P. Tytler and P. Calow, Eds.), Croom-Helm, London.

Campbell, R.C. 1984. Stocking of the Almond for salmon and sea trout improvement. Cramond Angling Club, Edinburgh.

Cantrelle, I. 1981. Etude de la migration et de la pêche des civelles (*Anguilla anguilla*) dans l'estuairé de la Gironde. Thèse III cycle, Universite Paris et Madam Curie.

Cantrelle, I. 1984. Les populations de civelles d'*Anguilla anguilla* (L.) en migration dans l'estuairé de la Gironde. Vie Milieu, **34**, 109-116.

Champ, W.S.T. 1968. A study of the eel population in the River Boyne system, with particular reference to growth, age and sexual differentiation. M.Sc. thesis, National University of Ireland, University College, Dublin.

Christensen, M. 1964. Burning of otoliths, a technique for age determination of soles and other fish. J. Cons. Perm. Int. Explor. Mer., **29**, 73-81.

Clegg, J. 1963. Pond and Stream life of Europe. Ysel Press Ltd., Deventer, Holland.

Conneely, J.J. and McCarthy, T.K. 1986. Ecological factors influencing the composition of the parasitic fauna of the European eel, *Anguilla anguilla* (L.), in Ireland. J. Fish. Biol., **28**, 207-219.

Craig, J.S. 1977. The body composition of adult perch, *Perca fluviatilis*, in Windermere, with reference to seasonal changes and reproduction. J. Anim. Ecol., **46**, 617-632.

Cripps, S.J. 1983. Growth variation in cultured European eels, *Anguilla anguilla* (L.). M.Sc. thesis, University of Southampton.

Curtis, E.J.C. and Curds, C.R. 1971. Sewage fungus in rivers in the United Kingdom: the slime community and its constituent organisms. Water Res., **5**, 1147-1159.

D'Ancona, U. 1950. Détermination et différenciation du sexe chez les poissons. Arch. Anat. Microsc. Morphol. Exp., **39**, 274-294.

D'Ancona, U. 1960. The life cycle of the Atlantic eel. Symp. Zool. Soc. Lond., **1**, 61-75.

Dave, G., Johansson, M.L., Larsson, A., Lewander, K. and Lidman, U. 1974. Metabolic haematological studies on the yellow and silver phases of European eel, *Anguilla anguilla*, L. - II. Fatty Acid composition. Comp.Biochem. Physiol., **47**B, 583-591.

Deelder, C.L. 1957. On the growth of eels in the Ijsselmeer. J. Cons. Perm. Int. Explor. Mer., **23**, 83-88.

Degani, G., Hahamu, H, Levanon, D., 1986. The relationship of eel *Anguilla anguilla* (L.) body size, lipid, protein, glucose, ash, moisture composition and enzyme activity (aldolase). Comp. Biochem. Physiol. A. Comp. Physiol., **84**, 739-745.

Degani, G. and Gallacher, M.L. 1985. Effects of dietary 17 -α- methyltestosterone and bovine growth hormone on growth and food conversion of slow- and fast-growing American elvers (*Anguilla rostrata*). Can. J. Fish. Aquat. Sci., **42**, 610-629.

Dekker, W. 1987. Regional variation in glass eel catches: an evaluation of multiple sample sites. EIFAC working party on eel, Bristol.

Dogiel, V.A., Petrushevski, G.K. and Polyanski, YuJ. 1958. Parasitology of Fishes, 1st English ed., 1961. Oliver and Boyd Ltd, Edinburgh.

Donaldson, E.M. 1981. The pituitary-interrenal axis as an indicator of stress in fish. In: Stress and Fish (A.D. Pickering, Ed.). Academic Press, London, pp.11-47.

Draganik, B. 1962. Food of eels from the Mazury Lake District. Zesz. Nauk. Wyzsz. Szk. Roln. Olsztyn, **13**, 141-167.

Eddy, F.B. 1981. Effects of stress on osmotic and ionic regulation in fish. In: Stress and Fish (A.D. Pickering, Ed.),. Academic Press, London, pp.77-102.

Egusa, S. 1979. Notes on the culture of the European eel (*Anguilla anguilla* (L.)) in Japanese eel-farming ponds. Rapp. Pv. Reun. Cons. Int. Explor. Mer., **174**, 51-58.

EIFAC (European Inland Fisheries Advisory Commission), 1970. Report on ammonia and inland fisheries. Technical paper no. 11, Rome.

Ezzat, A.E. and El Seraffy, S.S. 1977. Food of *Anguilla anguilla* in Lake Manzalah, Egypt. Mar. Biol. (Berl.), **41**, 287-291.

Fänge, R. and Groves, D.J. 1979. Digestion. In: Fish physiology (Hoar, W.S., Randall, D.J. and Brett, J.R. Eds). Vol. VIII. Academic Press, London, pp.161-260.

Fitter, R. and Manuel, R. 1986. Collins Field Guide to Freshwater Life. Collins, London.

Fontaine, M. et Tuzet, O. 1937. Sur la spermatogenese de l'anguille argentée. Arch. Zool. Exp. Gen., **78**.

Frost, W.E. 1945. The age and growth of eels (*Anguilla anguilla*) from the Windermere catchment area. Part 1 and Part 2. J. Anim. Ecol., **14**, 26-36 and 106-124.

Frost, W.E. 1946. Observations on the food of eels (*Anguilla anguilla*) from the Windermere catchment area. J. Anim. Ecol., **15**, 43-53.

Gallagher, M.L., Kane, E. and Beringer, R. 1984a. Effect of size on composition of the American eel, *Anguilla rostrata*. Comp. Biochem. Physiol., **78** A, 533-536.

Gallagher, M.L., Kane, E. and Courtney, J. 1984b. Differences in oxygen consumption and ammonia production among American elvers (*Anguilla rostrata*). Aquaculture, **40**, 183-187.

Ginetsinskaya, T.A. 1958. The lifecycles of fish helminths and the biology of their larval stages. In: Parasitology of Fishes (Dogiel, V.A., Petrushevski, G.K. and Polyanski, YuJ. Eds). Oliver and Boyd Ltd, Edinburgh.

Gousset, B. 1988. A note on eel research in Japan. Aquacult. Fish. Manage., **19**, 393-403.

Grimas, U. 1963. Reflections on the availability and utilization degree of bottom animals as fish food. Zool. Bidr. Uppsala, **35**, 497-503.

Gunnes, K., 1976. Effect of size grading young Atlantic salmon on subsequent growth. Aquaculture, **9**, 381-386.

Halsband, E. 1967. Basic principles of electric fishing. In: Fishing with electricity - its application to biology and management (R. Vibert Ed.), Fishing News (Books) Ltd., London.

Hartley, P.H.T. 1948. Food and feeding relationships in a community of freshwater fishes. J. Anim. Ecol., **17**, 1-14.

Hellawell, J.M. 1978. Biological surveillance of rivers: a biological monitoring handbook. Natural Environment Research Council, London.

Hughes, G.M. 1981. Effects of low oxygen and pollution on the respiratory systems of fish. In : Stress and Fish (A.D. Pickering, Ed.),. Academic Press, London, pp. 121-146.

Hussein, S.A. 1983. The Biology of the Freshwater eel *Anguilla anguilla*, L. in four tributaries of the River Tweed, Scotland. Ph.D thesis, University of Edinburgh.

Hussein, S.A. and Mills, D.H. 1982. The prevalence of 'cauliflower disease' of the eel, *Anguilla anguilla*, L., in tributaries of the River Tweed, Scotland. J. Fish Dis., **5**, 161-165

Hyatt, K.D. 1979. Feeding strategy. In: Fish Physiology, Vol VIII. (W.S. Hoar, D. J. Randall and J. R. Brett, Eds.), Academic Press, London, pp. 71-119.

Hynes, H.B.N. 1950. The food of the freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. J. Anim. Ecol., **19**, 36-58.

Hynes, H.B.N. 1976. The Ecology of Running Waters. Liverpool University Press.

Hyslop, E.J. 1980. Stomach contents analysis - a review of methods and their application. J. Fish Biol., **17**, 411-429.

Iles, T.D. 1974. The tactics and stragegy of growth in fishes. In: Sea Fisheries Research (F. R. Harden Jones Ed.), Elek Science, London.

Ingram, M.V. Pers. Comm. Marine Farm Limited, Stolford, Bridgewater, Somerset, England. (In addition: Fish Farming International, **15**, 6, 1.).

Iqbal, M. 1983. An introduction to solar radiation. Academic Press, Canada.

Ivlev, V.S. 1961. Experimental Ecology of the Feeding of Fishes. Translated from Russian by Douglas Scott. Yale University Press, New Haven.

Jobling, M. 1982. Some observations on the effects of feeding frequency on the food intake and growth of Plaice, *Pleuronectes platessa*, L. J. Fish Biol., **20**, 431-444.

Jobling, M. 1983. Effect of feeding frequency on food intake and growth of Arctic charr, *Salvelinus alpinus* (L). J. Fish Biol., **23**, 177-185.

Jobling, M. 1985. Physiological and social constraints on growth of fish with special reference to Arctic charr, *Salvelinus alpinus* (L). Aquaculture, **44**, 83-90.

Jobling, M. and Reinsnes, T-G. 1986. Physiological and social constraints on growth of Arctic charr, *Salvelinus alpinus*, L.: an investigation of factors leading to stunting. J. Fish Biol., **28**, 379-384.

Kastelein, P. 1983. Survival and growth of elvers (*Anguilla anguilla*) reared on expanded granulated diet. Aquaculture, **30**, 155-172.

Kennedy, C.R. 1984. The dynamics of a declining population of the acanthocephalan *Acanthocephalas clavula* in eels, *Anguilla anguilla* in a small river. J. Fish Biol., **25**, 665-77.

Kennedy, C.R. Pers. Comm. Department of Biological Sciences, University of Exeter.

Kennedy, M. and Fitzmaurice, P. 1969. Factors affecting the growth of coarse fish. The Fourth British Coarse Fish Conference.

Kennedy, C.R. and Lord, D. 1982. Habitat specificity of the acanthocephalan *Acanthocephalas clavula* (Dujardin, 1845) in eels *Anguilla anguilla* (L.). J. Helminthol., **56**, 121-129.

Kimura, M. and Ohta, T. 1971. Protein polymorphism as a phase of molecular evolution. Nature (Lond.), **229**, 467-469.

Knights, B. 1982. Body dimensions of farmed eels (*Anguilla anguilla* (L.)) in relation to condition factor, grading, sex and feeding. Aquacultural Engng., **1**, 297-310.

Knights, B. 1983. Food particle size preferences and feeding behaviour in warm water aquaculture of European eel *Anguilla anguilla* (L.). Aquaculture, **30**, 173-190.

Knights, B. 1984. Energetics and fish farming. In: Fish energetics: new perspectives (P. Tytler and P. Calow, Eds). Croom-Helm, London, pp. 309-340.

Knights, B. 1985. Feeding behaviour and fish culture. In: Nutrition and feeding in fish. (C.B. Cowey, A.M.Mackie and J.G. Bell, Eds.), Academic Press, London.

Knights, B. 1987. Agonistic behaviour and growth in the European eel, *Anguilla anguilla* L., in relation to warm-water aquaculture. J. Fish Biol., **31**, 265-276.

Knights, B. 1989. Effects of ammonia accumulation on metabolic rates and growth of European eel, *Anguilla anguilla* L., in relation to warm water aquaculture. Aquacult. Fish. Manage., 20, 111-118.

Koebele, B.P. 1985. Growth and the size hierarchy effect: an experimental assessment of three proposed mechanisms; activity differences, disproportional food acquisition, physiological stress. Environ. Biol. Fishes, **12**, 181-188.

Koehn, R.K., 1970. Genetic variation in the eel: a critique. Mar. Biol. (Berl.), **14**, 179-181.

Koops, H. and Kuhlmann, H. 1981. Annual variation of feeding and growth rate of eels farmed in thermal effluents of a conventional power station. In: Aquaculture in heated effluents and recirculation systems. Proc. of a world symp., Stavanger. Vol II (Tiews, K. Ed.) pp.205-211.

Krebs, J.R. 1978. Optimal foraging: decision rules for predators. In: Behavioural ecology: An evolutionary approach (J.R.Krebs and N.B.Davies Eds). Blackwell, Oxford, pp.23-63.

Kuhlmann, H. 1974. Experimental investigations of the influence of water temperature and qualitative food supply on the growth and sexual differentiation of the European eel (*Anguilla anguilla*). Doctoral dissertation, Faculty of Biology, University of Hamburg (Translated from German by Ministry of Agriculture, Fisheries and Food, London).

Kuhlmann, H. 1979. The influence of temperature, food, initial size and origin on the growth of elvers, *Anguilla anguilla* (L.). Rapp. Pv. Reun. Cons. Int. Explor. Mer., **174**, 59-63.

Kuhlmann, H. and Koops, H. 1980. New technology for rearing elvers in heated waters. EIFAC Symposium on new developments in the utilization of heated effluents and of recirculation systems for intensive aquaculture. No. E2, Stavanger.

Lagler, K.F. 1956. Freshwater Fishery Biology. Wm. C. Brown, Dubuque, Iowa.

Leclerc, E. and Devlaminck, F. 1955. Les assais de toxicologie des poissons et la qualite des eaux. Bull. Centr. Belg. Etude Doc. Eaux., **30**, 111-113.

Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J. Anim. Ecol., **20**, 210-219.

Liewes, E.W. 1978. Eel farming in Europe. Fish Farming Int., **5**, (3) 8-13.

Love, R.M. 1970. The Chemical Biology of Fishes. Academic Press, London.

Love, R.M. 1980. The Chemical Biology of Fishes: Vol. 2, advances 1968-1977. Academic Press, London.

McDonald, A.L., Heimstra, N.W. and Damkot, D.K. 1968. Social modification of agonistic behaviour in fish. Anim. Behav., **16**, 437-441.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D. 1981. Animal Nutrition, third ed. Longman, London.

Macan, T.T. 1958. Methods of sampling the bottom fauna in stony streams. Mitt. Int. Ver. Theor. Angew. Limnol., **8**, 1-21.

Mackie, A.M. and Mitchell, A.I. 1983. The chemical nature of feeding stimulants for the juvenile European eel, *Anguilla anguilla* (L). J. Fish. Biol., **22**, 425-430.

Mackie, A.M. and Mitchell, A.I. 1985. Identification of gustary feeding stimulants for fish applications in aquaculture. In: Nutrition and feeding in fish (C.B. Cowey, A.M. Mackie and J.G.Bell, Eds). Academic Press, London.

Malecha, S.R., Masuno, S. and Onizuka, D. 1984. The feasibility of measuring the hereditability of growth pattern variation in juvenile freshwater prawns *Machrobrachium rosenbergii* (de Man). Aquaculture, **38**, 347-363.

Mann, R.H.K. 1976. Observations on the age, growth, reproduction and food of the pike *Esox lucius* L. in two rivers in southern England. J. Fish Biol., **8**, 179-197.

Mann, R.H.K. and Orr, D.R.O. 1969. A preliminary study of the feeding relationships of fish in a hard-water and soft-water stream in southern England. J. Fish Biol., **1**, 31-44.

Matsui, I. 1952. Studies on the morphology, ecology and pond-culture of the Japanese eel (*Anguilla japonica*). J. Shimonoseki Coll. Fish., **2**(2), 1-245.

Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc., **106**, 201-212.

Mazeaud, M.M. and Mazeaud, F. 1981. Adrenergic responses to stress in fish. In: Stress and fish (A.D.Pickering, ed.). Academic Press, London, pp. 49-75.

Micheler, G. 1967. Biologische Untersuchungen an den Aalen des Chiemsees. Ph.D. thesis, Ludwig-Maximilians Univ., München.

Morgan, N.C. and Egglishaw, H.J. 1965. A survey of the bottom fauna of streams in the Scottish Highlands. Part I. Composition of the fauna. Hydrobiologia, **25**, 181-211.

Moriarty, C. 1972a. Eel research 1965-1971. Fishery leaflet No. 43, Fisheries Division, Dublin.

Moriarty, C. 1972b. Studies of the eel *Anguilla anguilla* in Ireland 1: in the lakes of the Corrib System. Ir. Fish. Invest. Ser. A (Freshwater), **10**.

Moriarty, C. 1973a. Studies of the eel *Anguilla anguilla* in Ireland 2. In Lough Conn, Lough Gill and North Cavan Lakes. Ir. Fish. Invest. Ser. A (Freshwater), **13**, 1-13.

Moriarty, C. 1973b. A technique for examining eel otoliths. J. Fish Biol., **5**, 183-4.

Moriarty, C. and Steinmetz, B. 1979. On Age Determination of Eel. Rapp. Pv. Reun. Cons. Int. Explor. Mer., **174**, 70-74.

Moriarty, C. 1983. Age determination and growth rate of eels *Anguilla anguilla* (L). J. Fish Biol., **23**, 257-264.

Moriarty, C. 1986. Riverine migration of young eels *Anguilla anguilla* (L.). Vie Milieu, **36**, 266.

Moriarty, C. 1987a. The eel in Ireland. Royal Dublin Society, Went Memorial Lecture, 17 November.

Moriarty, C. 1987b. Observations on elver catches at European stations. EIFAC, working party on eel, Bristol.

Moriarty, C., Dekker, W. and Elie, P. 1987. Elver stocks: a critical assessment of previous studies and proposals for future monitoring. EIFAC working party on eel, Bristol.

Muir, J.F. 1976. How filters improve water quality for fish farmers. Fish Farming Int., Sept. 1976, 35-38.

Nicol, J.A. 1963. Some aspects of photoreception and vision in fishes. Adv. Mar. Biol., **1**.

Nikolsky, G.V., 1963. The Ecology of Fishes. Academic Press, London. Translated from Russian.

Noakes, D.L. and Leatherland, J.F. 1977. Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). Environ. Biol. Fishes, **2**, 131-136.

Ochiai, A., Tanaka, T. and Umet, S. 1981. Change in the chemical composition of the body of Japanese eel *Anguilla japonica*: fat content and fatty acid composition. Rep. USA. Mar. Biol. Inst. Kochi Univ., **7**, 67-72.

Oosten, V. 1944. Factors affecting the growth of fish. Trans. 9th North American wildlife conference (E.M. Quce Ed.). Am. Wildlife Inst., Washington, pp. 177-183.

Opuszynski, K. 1963. Attempts at investigating elvers (*Anguilla anguilla*) in Polish inland waters. Ekol. Pol. Ser. A, **2**, 59-85.

Otwell, W.S. and Rickards, W.L. 1982. Cultured and wild American eels, *Anguilla rostrata* fat content and fatty acid composition. Aquaculture, **26**, 67-76.

Pandian, T.J. and Vivekanandan, E. 1985. Energetics of feeding and digestion. In: Fish Energetics New Perspectives (P. Tytler and P. Calow, Eds). Croom-Helm, London, pp. 99-124.

Passakas, T. 1976. Further investigation on the chromosomes of *Anguilla anguilla* . Folia Biol. (Cracow), **24** (2), 239-244.

Peters, G., Delventhal, H. and Klinger, H. 1980. Physiological and morphological effects of social stress in the eel, (*Anguilla anguilla*). Arch. Fishchereiwiss, **30**, 157-180.

Peters, G. 1982. The effect of stress on the stomach of the European eel *Anguilla anguilla*. J. Fish Biol., **21**, 497-512.

Peters, G., Faisal, M., Lang, T. and Ahmad, I. 1988. Stress caused by social interaction and its effect on susceptibility to *Aeromonas hydrophila* infection in rainbow trout *Salmo gairdneri*. Dis. Aquat. Org., **4**(2), 83-89.

Pickering, A.D. 1981 (Ed.) Stress and Fish. Academic Press, London.

Pickering, A.D., Pottinger, T.G. and Christie, P. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. J. Fish. Biol., **20**, 229-242.

Pitcher, T.J. and Hart, J.B. 1982. Fisheries Ecology. Croom-Helm, London.

Poupard, C.J. 1978. Therapy of Fish Diseases. In: Fish Pathology (R.J.Roberts, Ed.), Balliere Tyndall, University Press, Aberdeen.

Poxton, M.G. and Allouse, S.B. 1987. Cyclical fluctuations in ammonium and nitrite-nitrogen resulting from the feeding of turbot, *Scophthalmus maximus* (L.), in recirculating systems. Aquacultural Engng., **6**, 301-322.

Purdom, C.E. 1974. Variation in fish. In: Sea Fisheries Research (F. R. Harden Jones Ed.), Elek Science, London.

Rasmussen, G. and Therkildsen, B. 1979. Food, Growth and Production of *Anguilla anguilla* L. in a small Danish stream. Rapp. Pv. Reun. Cons. Int. Explor. Mer., **174**, 32-40.

Ricker, W.E. 1968. Methods for assessment of fish production in fresh waters. IBP Handbook No. 3. Blackwell Scientific, London.

Ricker, W.E. 1979. Growth rates and models. In: Fish Physiology Vol. VIII (Hoar, W.S., Randall, D.J. and Brett, J.R. Eds). Academic Press, London.

Riley, J.D. 1966. Liquid latex marking technique for small fish. J. Cons. Perm. Int. Explor. Mer., **30**, 354-357.

Rodino, E. and Comparini, A. 1978. Genetic variability in the European eel *Anguilla anguilla* L. In: Marine Organisms, Genetics, Ecology and Evolution. (Battaglia, B. and Beardmore, J.A. Eds), NATO Conf. Series IV(2), New York Plenum Press pp. 389-424.

Rossi, R. and Villani, P. 1980. A biological analysis of eel catches, *Anguilla anguilla* L., from the lagoons of Lesina and Varano, Italy. J. Fish Biol., **16**, 413-423.

Royal Commission on Sewage Disposal, 1912.

Sadler, K. 1979. Effects of temperature on the growth and survival of the European eel, *Anguilla anguilla* L. J. Fish Biol., **15**, 499-507.

Sadler, K. 1981. Toxicity of ammonia to the European eel (*Anguilla anguilla*). Aquaculture, **26**, 173-182.

Sarti, M., Giorgetti, G. and Brisinelle, W. 1985. A new problem for intensive eel rearing in Italy: *Anguillicola australiensis*. EAFP, Montpellier.

Schmidt, J. 1906. Contributions on the life-history of the Eel. (*Anguilla vulgaris*, Flem.) Rapp. Pv. Reun. Cons. Perm. Int. Explor. Mer, 5(4), 137-274.

Schmidt, J. 1912. Danish researches in the Atlantic and Mediterranean on the life-history of the freshwater eel (*Anguilla vulgaris*, Turt.) with notes on other species. Int. Revue Ges. Hydrobiol. Hydrogr., **5**, 317-342.

Schmidt, J. 1922. The breeding places of the eel. Phil. Trans. R. Soc. Lond. (Ser.B), **211**, 179-209.

Schreck, C.B. and Lorz, H.W. 1978. Stress response of the Coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Board. Can., **35**, 1124-1129.

Schreck, C.B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. In: Stress and Fish (A.D.Pickering, ed.). Academic Press, London, pp. 295-321.

Seyle, H. 1956. The Stress of Life. McGraw-Hill, New York.

Seymour, E.A. 1984. Feeding biology, growth and behaviour of the European eel, *Anguilla anguilla* (L). in relation to warm water aquaculture. Ph.D. thesis, Polytechnic of Central London.

Seymour, E.A. 1989. Devising optimum feeding regimes and temperatures for warm water culture of eel, *Anguilla anguilla* L. Aquacult. Fish. Manage., **20**, 1, 129-142.

Siegel, S. 1956. Nonparametric Statistics for the Behavioural Sciences. McGraw-Hill, New York.

Sinha, V.R.P. 1965. Studies on the biology of the freshwater eel *Anguilla anguilla* L. Ph.D. thesis, University of Liverpool.

Sinha, V.R.P. and Jones, J.W. 1966. On the sex and distribution of the freshwater eel (*Anguilla anguilla*). J. Zool. (Lond.), **150**, 371-385.

Sinha, V.R.P. and Jones, J.W. 1967a. On the age and growth of the freshwater eel (*Anguilla anguilla*). J. Zool. (Lond.), **153**, 99-117.

Sinha,V.R.P. and Jones, J.W. 1967b. On the food of the fresh water eels and their feeding relationship with the salmonids. J. Zool. (Lond.), **153**, 119-137.

Sinha, V.R.P. and Jones, J.W. 1975. The European Freshwater Eel. Liverpool University Press, 1975.

Sloane, R.D. 1984. Distribution and abundance of freshwater eels (*Anguilla* spp.) in Tasmania. Aust. J. Mar. Freshwater Res., **35** (4), 463-470.

Smart, G.R. 1981. Aspects of water quality producing stress in intensive fish culture. In: Stress and Fish (A.D.Pickering, Ed.), Academic Press, London, pp. 277-293.

Smith, L. 1982. Introduction to Fish Physiology. TFM Publications, New Jersey.

Sokal, R.R. and Rohlf, F.J. 1969. Biometry, 2nd ed. W.H.Freeman, San Francisco.

Somero, G.N. and Soule, M. 1974. Genetic variation in marine fishes as a test of the niche-variation hypothesis. Nature (Lond.), **249**, 570-572.

Sommerville, C. Pers. Comm. Institute of Aquaculture, University of Stirling.

Sparre, P. 1979. Some necessary adjustments for using the common methods in eel assessment. Rapp. Pv. Reun. Cons. Int. Explor. Mer., **174**, 41-44.

Strauss, R.E. 1979. Reliability estimates for Ivlev's electivity index, the forage ratio, and proposed linear index of food selection. Trans. Am. Fish. Soc., **108**, 344-352.

Strubberg, A. 1913. The metamorphosis of elvers as influenced by outward conditions. Meddr. Komm. Danm. Fisk. Havunders. Ser. Fiskeri, **4**(3), 1-11.

Strubberg, A.C. 1923. Elvers from North and South Europe. Rep. Dan. Oceanogr. Exped. Mediterr., **3**, 1-23.

Sumner, J.L., Hopkirk, G. 1976. Lipid composition of New Zealand eels. J. Sci. Food Agric., **27**, 933-938.

Tacon, A.G.J., Stafford, E.A. and Edwards, C.A. 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for Rainbow trout. Aquaculture, **35**, 187-199.

Takeda, M., Takii, K. and Matsui, K. 1984. Identification of feeding stimulants for juvenile eel. Bull. Jpn. Soc. Sci. Fish., **59**(4), 645-651.

Tesch, F.-W., 1973. Der Aal. Paul Parey, Hamburg and Berlin. The Eel. Biology and Management of Anguillids. Revised and updated, English language edition 1977, Chapman and Hall.

Tesch, F.-W. 1985. A review on the competitive situation of the eel in relation to other species. EIFAC working party on eel, Perpignan.

Thompson, D'Arcy, W. 1942. On growth and form. Vols. 1 and 2. Cambridge University Press.

Thurow, F. 1957. Beiträge zur Biologie des Flußoaks *Anguilla vulgaris* Turt. Ph.D. thesis, Universitat Kiel.

Törlitz, H. 1922. Contribution to the anatomy and development of the river eel related to its eel species question. Z. Fisch., **21**, 1-48.

Trussel, R.P. 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Board. Can., **29**, 1505-1507.

Usui, A. 1974. Eel Culture. Fishing News (Books), West Byfleet, U.K.

Uzmann, J.R., Paulik, G.J. and Hayduk, S.H. 1965. Experimental hexamitiasis in juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). Trans. Am. Fish. Soc., **94**, 53-61.

Varley, M.E. 1967. British Freshwater Fishes: factors affecting their distribution. Fishing News (Books), West Byfleet, U.K.

Völlestad, L.A. 1985. Age determination and growth of yellow eels, *Anguilla anguilla* (L.), from a brackish water, Norway. J. Fish Biol., **26**, 521-525.

Völlestad, L.A., Lecomte-Finiger, R.L. and Steinmetz, B. 1988. Age determination of *Anguilla anguilla* and related species. EIFAC Occasional Paper, 21.

Wankowski, J.W.J. and Thorpe, J.E. 1976. The role of food particle size in the growth of juvenile Atlantic salmon *Salmo salar*. J. Fish Biol., **14**, 351-370.

Weatherley, A.H. 1972. Growth and Ecology of Fish Populations. Academic Press, London.

Webber, H.H. and Huguenin, H.E. 1979. Fish feeding technologies. In: Finfish Nutrition and Fishfeed Technology, Vol. 1. (J.E. Halver and K. Tiews, Eds) Heinemann, Berlin, pp. 297-316.

Weber, D.D. and Ridgeway, G.J. 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. Prog. Fish-Cult., **24**(4), 150-155.

Wedemeyer, G.A. and McLeay, D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. In: Stress and Fish (A.D. Pickering, Ed.) Academic Press, London, pp. 247-275.

Westernhagen, H. von, Rosenthal, H., Dethlefsen, V., Ernst, W., Harms, U. and Hansen, P.D. 1981. Bio-accumulating substances and reproductive success in Baltic flouder *Platicthys flesus*. Aquat. Toxicol. (Amst.), **1**, 85-99.

Wickins, J.F. 1983. Speed-up for slow eels. Fish farmer, **6**(2), 24-25.

Wickins, J.F. 1985. Growth variability in individually confined elvers, *Anguilla anguilla*. J. Fish Biol., **27**, 469-478.

Wickins, J.F. 1987. Effects of size, culling and social history on growth of cultured elvers, *Anguilla anguilla* (L.). J. Fish Biol., **31**, 71-82.

Willemse, J.J., Markus-Silvis, L. and Ketting, G.H. 1984. Morphological effects of stress in cultured elvers, *Anguilla anguilla*. Aquaculture, **36**, 193-201.

Williams, G.C., Koehn, R.K., Mitton, J.B. 1973. Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. Evolution, **27**, 192-204.

Wilson, E.O. 1975. Sociobiology: the New Synthesis. Belknap Press, Harvard University.

Windell, J.T. and Bowen, S.H. 1978. Methods for study of fish diets based on analysis of stomach contents. In: Methods for assessment of fish production in freshwaters. 3rd edn. (T. Bagnel, Ed.). Blackwell Scientific, London, pp. 219-226.

Woodiwiss, F.S. 1964. The biological system of stream classification used by the Trent River Board. Chemistry and Industry II, pp. 443-447.

Yamamoto, K. and Yamauchi, K. 1974. Sexual maturation of Japanese eel and production of eel larvae in the aquarium. Nature (Lond.), **251**, 220-222.

APPENDIX 2.1 Forth River Purification Board water quality values for the River Almond in 1985 and 1986 (monthly means)

Parameter	Unit	1985	1986
Temperature	°C	9.2 (1.5-15.5)	8.5 (1-20.0)
pH	unit	7.6 (7.1-7.9)	7.6 (6.9-7.8)
Conductivity	μs^{-cm}	613 (284-910)	560 (205-980)
Total alkalinity	as $CaCO_3$	134 (80-175)	110 (45-225)
Total hardness	as $CaCO_3$	249 (164-330)	230 (84-550)
Chloride	$mg.l^{-1}$	63 (24-98)	60 (21-137)
Suspended solids	$mg.l^{-1}$	9.3 (4.0-17.0)	38 (4-186)
Dissolved oxygen	$mg.l^{-1}$	10.3 (7.0-15.0)	9.7 (6.7-12.0)
Oxygen saturation	%	85.9 (66.1-137.7)	82.5 (57.6-105.0)
B O D	$mg.l^{-1}$	4.0 (2.6-6.5)	4.0 (2.2-5.9)
B O D + A T U	$mg.l^{-1}$	3.0 (0.6-5.3)	3.2 (1.7-4.6)
C O D	$mg.l^{-1}$	39.0 (24.0-50.0)	43.3 (9.0-87.0)
Total ammonia	$mg.l^{-1}$	1.20 (0.15-3.90)	1.17 (0.21-3.1)
Nitrite	$mg.l^{-1}$	0.20 (0.06-0.35)	0.21 (0.04-0.94)
Nitrate	$mg.l^{-1}$	4.41 (3.10-5.60)	4.1 (2.3-7.2)
A S D	$mg.l^{-1}$	-	0.23 (0.12-0.36)
Iron	$mg.l^{-1}$	0.89 (0.24-1.72)	1.8 (0.14-7.3)
Faecal coliforms	-100ml	-	12025 (7500-15600)
Total coliforms	-100ml	-	45250
Faecal streptococci	-100ml	-	1530 (290-2340)
Salmonella		-	$^{+}$ve

Key

B O D Biological oxygen demand

A T U Allyl-thio-urea (nitrification inhibitor)

C O D Chemical oxygen demand

A S D Anionic synthetic detergent as Manoxol O T

APPENDIX 2.2 Frequency of occurrence of food organisms in intestines of eels at Site 1 and Site 2 (n=number of intestines, f=frequency of occurrence, L=larva, P=pupae, A=adult)

Food organism	Site1		Site2	
	n	f	n	f
Slavina appendiculata	61	18.7	13	9.8
Nais spp.	43	13.2	10	7.5
Stylaria lacustris	22	6.7	7	5.3
Tubifex	5	1.5	7	5.3
Herpobdella octoculata	1	0.3	-	-
Asellus aquaticus	58	17.7	19	14.3
Odonata	-	-	1	0.8
Plecoptera	1	0.3	-	-
Ephemeroptera	23	7.0	6	4.5
Hydroporus sp. (L)	2	0.6	4	3.0
Hydroporus sp. (A)	4	1.2	1	0.8
Lepidoptera (L)	1	0.3	2	1.5
Trichoptera	7	2.1	1	0.8
Tipulid (L)	1	0.3	-	-
Simulium sp. (L)	4	1.2	2	1.5
Chironomid spp. (L)	65	19.9	33	24.8
Chironomid spp. (P)	15	4.6	13	9.8
Chironomid spp. (A)	2	0.6	4	3.0
Forcipomyia sp.	2	0.6	-	-
Chironomus sp.	-	-	2	1.5
Terrestrial Insecta	4	1.2	1	0.8
Hydracarina	1	0.3	-	-
Limnaea pereger	4	1.2	5	3.8
Aplecta fontinalis	-	-	1	0.8
Ancylastrum fluviatile	2	0.6	-	-
Anguilla anguilla	2	0.6	-	-

APPENDIX 7.1 Change in weight (mg) of glass eels transferred to culture in May, June and July (median weight (Q2), mean weight (M) and standard deviation (sd), r=replicate)

	r	Days	1	12	24	36	48	60	72	84
May	1	Q2	250	346	456	507	549	499	613	619
		M	248	342	465	524	540	521	709	770
		sd	28	38	83	127	149	189	343	472
	2	Q2	245	307	393	411	483	416	382	-
		M	245	312	427	462	635	596	612	1187
		sd	34	44	117	190	358	433	581	1083
	3	Q2	251	336	465	483	508	488	513	546
		M	253	327	439	462	521	493	556	587
		sd	36	47	103	148	135	164	252	341
June	1	Q2	228	257	339	456	-	-	-	-
		M	230	257	339	459	-	-	-	-
		sd	37	43	64	105	-	-	-	-
	2	Q2	231	230	272	368	-	-	-	-
		M	233	231	299	385	-	-	-	-
		sd	39	40	73	103	-	-	-	-
	3	Q2	222	245	307	388	-	-	-	-
		M	226	241	315	389	-	-	-	-
		sd	30	32	66	96	-	-	-	-
July	1	Q2	217	348	405	398	-	-	-	-
		M	232	337	392	398	-	-	-	-
		sd	44	48	83	79	-	-	-	-
	2	Q2	234	312	413	406	-	-	-	-
		M	248	323	387	379	-	-	-	-
		sd	57	55	69	66	-	-	-	-
	3	Q2	214	291	364	370	-	-	-	-
		M	230	301	379	377	-	-	-	-
		sd	38	62	115	125	-	-	-	-

APPENDIX 8.1 ***(Experiment 8.1)*** **Change in weight (mg) of elvers on a diet of tubifex in different physical form (r=replicate, median (Q2), mean (M), standard deviation (sd))**

Diet	r	Day	1	7	14	21	28
Live	1	Q2	233	300	395	490	604
		M	244	305	384	494	600
		sd	29	48	73	113	168
	2	Q2	238	282	370	542	604
		M	236	275	352	489	630
		sd	22	43	83	153	218
	3	Q2	231	272	361	476	555
		M	230	279	363	471	553
		sd	24	47	67	104	143
Chopped	1	Q2	229	245	299	346	376
		M	234	239	302	345	381
		sd	25	42	46	40	49
	2	Q2	234	273	353	432	476
		M	238	273	340	422	472
		sd	30	46	61	93	123
	3	Q2	236	280	336	542	541
		M	238	272	348	529	529
		sd	26	44	74	102	99
Dead	1	Q2	225	252	293	334	357
		M	240	253	286	316	347
		sd	35	40	54	68	67
	2	Q2	226	226	288	283	308
		M	231	231	275	298	327
		sd	23	44	76	103	117
	3	Q2	226	224	232	270	316
		M	234	226	252	272	305
		sd	25	36	46	64	69
Dried	1	Q2	220	225	243	267	278
		M	232	224	237	280	282
		sd	28	36	53	44	64
	2	Q2	218	201	227	254	255
		M	227	209	226	251	256
		sd	22	29	36	18	29
	3	Q2	231	205	209	233	285
		M	238	215	239	278	292
		sd	29	39	60	82	100

APPENDIX 8.2 *(Experiment 8.2)* **Change in weight (mg) of elvers in gravel and non-gravel (median (Q2), mean (M), standard deviation (sd), 1=150-199mg, 2=200-249mg, 3=250-299mg, 4=300-349mg, 5=350-399mg)**

		Day	1	7	14	21	28	35
Gravel	1	Q2	189	255	324	418	543	696
		M	185	255	323	422	533	701
		sd	12	32	54	74	111	158
	2	Q2	227	290	376	521	670	838
		M	223	284	377	480	665	854
		sd	16	41	71	106	169	231
	3	Q2	278	369	465	594	840	963
		M	278	370	474	604	812	1008
		sd	15	48	76	118	191	306
	4	Q2	323	405	489	646	860	1182
		M	326	414	527	681	909	1220
		sd	11	63	128	201	314	469
	5	Q2	365	494	646	798	1009	1339
		M	374	491	624	801	1053	1386
		sd	25	42	111	197	312	424
Non-gravel	1	Q2	191	235	313	382	457	472
		M	186	239	302	362	424	446
		sd	11	19	32	69	107	133
	2	Q2	221	280	333	410	450	573
		M	222	284	354	427	511	612
		sd	8	37	89	160	247	319
	3	Q2	266	335	360	396	395	391
		M	268	337	365	403	402	427
		sd	17	37	61	86	105	123
	4	Q2	321	414	532	562	663	680
		M	321	405	499	608	687	715
		sd	12	46	94	150	173	222
	5	Q2	372	463	556	673	767	897
		M	376	467	571	702	827	963
		sd	20	29	61	121	219	322

APPENDIX 9.1 ***(Experiment 9.1)*** **Change in median weight (Q2), mean weight (M) and standard deviation (sd)**

					Cross-over x1			Cross-over x2		
Tank	Week	0	2	3	4	5	6	7	8	9
ABA1	Q2	226	381	464	518	593	725	1022	1277	1614
	M	226	378	447	536	648	821	1173	1530	1963
	sd	15	52	87	99	158	261	430	675	1000
ABA2	Q2	219	383	466	571	621	748	990	1265	1517
	M	220	373	464	548	611	750	1014	1355	1741
	sd	13	59	91	138	215	305	448	642	847
BAB1	Q2	-	-	383	507	661	885	863	990	1081
	M	-	-	371	477	617	803	797	916	1020
	sd	-	-	67	126	177	225	208	257	282
BAB2	Q2	-	-	370	494	608	729	676	730	813
	M	-	-	365	461	585	655	612	675	764
	sd	-	-	73	129	171	211	182	206	260
CDC1	Q2	177	191	170	167	207	262	301	348	418
	M	178	198	178	182	206	273	307	363	445
	sd	13	30	41	69	93	109	128	127	114
CDC2	Q2	217	217	213	234	275	351	383	420	475
	M	221	225	218	242	289	346	376	410	473
	sd	15	41	45	70	85	113	122	133	150
DCD1	Q2	273	236	269	282	302	352	424	508	591
	M	274	246	268	270	297	357	423	505	603
	sd	16	51	80	77	91	83	102	126	163
DCD2	Q2	271	265	308	318	335	394	475	560	694
	M	276	288	323	313	324	383	467	618	766
	sd	18	70	120	120	129	132	165	171	250

APPENDIX 9.2 Length (L), weight (W) and proximate analysis of individual fish in *Experiment 9.2*

Day	1.1 L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1	10.8	10.4	10.3	10.5	10.6	1.66	1.24	1.27	1.17	1.36	59.09	33.96	6.95
12	11.0	10.4	10.4	10.2	10.4	1.59	1.11	1.32	1.08	1.37	70.38	18.08	11.54
24	11.0	10.5	10.6	10.3	10.9	1.56	0.98	1.38	1.19	1.43	60.71	30.32	8.97
36	11.0	10.6	10.8	10.5	10.9	1.45	0.87	1.39	1.26	1.38	62.99	28.68	8.33
48	11.2	10.7	10.9	10.9	11.1	1.51	0.91	1.42	1.33	1.43	61.42	31.26	7.32
60	11.1	10.6	10.9	10.7	11.0	1.42	0.83	1.26	1.21	1.35			
72	11.3	10.7	11.1	10.8	11.2	1.46	0.84	1.12	1.10	1.41			

Day	1.2 L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1	10.1	10.2	12.1	9.8	12.3	1.11	1.20	2.21	1.15	2.08	56.79	34.84	8.37
12	10.0	10.2	12.3	9.8	12.5	0.96	1.14	2.09	1.15	2.06	-	-	-
24	10.0	10.2	12.3	9.9	13.1	0.83	1.12	2.08	1.14	2.48	-	-	-
36	12.4	10.2	12.2	-	13.6	1.74	1.06	2.05	-	2.71	-	-	-
48	12.5	10.2	12.3	-	14.0	1.68	1.06	2.05	-	2.74	62.65	28.45	8.90
60	12.2	10.3	12.3	-	13.9	1.50	0.82	1.79	-	2.45			
72						1.41				2.50			

Day	2.1 L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1	12.7	15.9	13.2	13.1	14.8	2.76	4.86	3.10	3.06	3.79	-	-	-
12	12.8	16.3	13.3	-	15.5	2.49	4.64	2.94	-	4.43	-	-	-
24	12.8	-	13.3	-	15.9	2.57	-	2.91	-	4.75	-	-	-
36	12.9	-	13.2	-	16.5	2.66	-	2.76	-	4.99	-	-	-
48	-	-	13.2	-	17.0	-	-	2.66	-	5.31	54.04	40.11	5.85
60	-	-	13.2	-	17.5	-	-	2.28	-	5.80			
72	-	-	-	-	17.9	-	-	-	-	6.05			

Day	2.2 L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1	10.8	9.7	10.3	12.0	12.4	1.45	1.00	1.29	2.49	2.61	48.27	45.16	6.57
12	10.9	9.6	10.3	12.1	12.4	1.28	0.88	1.10	2.34	2.67	58.69	33.55	7.76
24	10.7	9.5	10.4	12.2	12.8	1.21	0.79	1.09	2.21	2.59	57.49	34.45	8.06
36	10.8	9.6	10.5	12.2	13.0	1.10	0.78	1.07	2.09	2.45	54.34	38.56	7.10
48	10.8	9.7	10.5	12.2	13.0	1.06	0.73	1.14	2.03	2.35	54.17	37.00	8.83
60	10.9	9.7	10.7	12.2	13.0	1.03	0.78	1.20	2.08	2.34			
72	10.9	9.8	10.8	12.4	13.2	1.02	0.82	1.19	2.02	2.35			

APPENDIX 9.2 continued. Length (L), weight (W) and proximate analysis of individual fish in *Experiment 9.2*

Day	3.1	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		12.7	12.3	13.7	12.5	12.3	2.68	2.69	3.33	2.31	2.12	65.57	27.67	27.67
12		12.8	13.2	14.4	13.0	12.8	2.69	3.31	3.59	2.66	2.30	56.49	36.53	36.53
24		13.0	13.6	14.6	13.4	12.9	2.64	3.36	3.64	2.78	2.33	58.72	33.94	33.94
36		13.1	14.2	15.1	13.7	12.8	2.62	3.81	3.93	2.96	2.19	62.56	29.70	29.70
48		13.1	14.5	15.6	14.0	13.0	2.54	3.98	4.44	3.21	2.19	-	-	-
60		13.1	14.7	16.7	14.4	12.8	2.70	4.14	5.29	3.60	2.51			
72			15.0	-	15.0	-	-	4.11	-	4.02	-			

Day	3.2	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		10.2	9.5	9.3	9.2	9.5	1.10	1.12	1.11	0.97	1.13	70.94	19.78	9.28
12		10.4	9.4	9.8	9.0	9.9	1.21	1.06	1.31	0.79	1.25	58.85	33.28	7.87
24		10.6	9.6	10.1	8.8	10.0	1.15	1.04	1.40	0.65	1.20	53.46	39.92	6.62
36		10.6	9.6	10.3	8.8	10.1	1.12	1.02	1.40	0.57	1.27	69.01	20.28	10.72
48		10.9	9.7	10.5	8.8	10.7	1.17	0.99	1.49	0.53	1.43	57.92	35.00	7.08
60		11.3	9.7	10.7	8.8	11.0	1.38	1.03	1.63	0.51	1.63			
72		11.6	9.8	11.0	9.0	11.5	1.43	1.06	1.66	0.52	1.73			

Day	4.1	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		10.0	9.6	9.1	9.7	10.0	1.04	1.12	0.87	0.81	1.18	61.82	30.16	8.02
12		9.8	9.9	9.2	9.6	10.0	0.98	1.31	0.96	0.74	1.27	52.84	41.14	6.02
24		9.8	10.1	9.5	9.7	10.1	0.91	1.32	0.99	0.73	1.20	57.24	36.55	6.21
36		9.8	10.3	9.5	9.6	10.2	0.86	1.34	0.99	0.64	1.13	-	-	-
48		9.9	10.7	9.6	9.7	10.2	0.82	1.47	0.98	0.68	1.05	60.23	31.98	7.79
60		9.9	10.9	9.6	9.8	10.2	0.81	1.50	1.04	0.75	1.06			
72		10.0	11.2	9.8	-	10.3	0.81	1.53	1.05	-	1.08			

Day	4.2	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		10.4	12.6	11.0	11.4	14.6	1.28	1.94	1.55	1.75	3.66	-	-	-
12		10.5	12.5	11.2	11.5	15.6	1.27	2.09	1.60	1.64	4.61	-	-	-
24		10.4	12.5	11.2	11.4	16.0	1.23	2.01	1.48	1.54	4.46	-	-	-
36		10.5	12.5	11.3	11.5	16.6	1.28	1.86	1.66	1.71	4.92	-	-	-
48		-	12.5	-	-	17.2	-	2.19	-	-	5.44	60.23	31.97	7.29
60		-	-	-	-	17.6	-	-	-	-	6.12			
72		-	-	-	-	18.0	-	-	-	-	6.24			

APPENDIX 9.2 continued. Length (L), weight (W) and proximate analysis of individual fish in *Experiment 9.2*

Day	5.1	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		10.3	12.6	11.0	10.5	12.1	1.34	2.65	1.92	1.35	2.37	-	-	-
12		10.3	12.7	11.0	10.6	12.2	1.22	2.51	1.60	1.35	2.20	58.80	33.92	7.28
24		10.3	12.7	11.0	10.5	12.2	1.16	2.32	1.45	1.32	1.87	-	-	-
36		10.4	12.8	11.1	10.5	12.2	1.08	2.23	1.29	1.24	1.87	-	-	-
48		10.3	12.8	10.9	-	12.2	1.04	2.28	1.22	-	1.90	60.50	31.90	7.60
60		10.2	12.8	-	-	12.1	1.18	2.25	-	-	1.95			
72		-	12.9	-	-	12.2	-	2.24	-	-	1.99			

Day	5.2	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		12.8	9.6	9.9	9.4	12.4	2.90	1.05	1.14	0.82	2.29	59.17	33.67	7.16
12		14.2	9.8	10.1	9.4	12.3	3.55	1.12	1.22	0.83	2.29	58.77	34.60	6.64
24		14.2	9.8	10.1	9.5	12.3	3.23	1.06	1.19	0.78	2.16	56.78	36.93	6.28
36		14.3	9.8	10.1	9.4	12.4	3.19	1.04	1.08	0.69	2.13	59.64	31.41	8.94
48		14.3	9.9	10.1	9.5	12.5	3.30	1.02	1.03	0.66	2.17	63.98	28.58	7.43
60		14.5	10.0	10.1	9.5	12.8	3.38	1.10	1.08	0.63	2.53			
72		14.8	10.1	10.2	9.5	13.0	3.47	1.15	1.06	0.60	2.85			

Day	6.1	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5	protein	lipid	ash
1		11.8	11.9	11.8	10.8	13.6	1.45	1.68	2.10	1.60	3.35	54.16	38.99	6.85
12		11.9	11.9	11.8	10.8	13.9	1.47	1.62	1.86	1.42	3.20	59.23	32.73	8.04
24		11.9	11.8	11.7	10.7	13.7	1.46	1.50	1.71	1.25	2.81	58.69	33.14	8.17
36		11.9	11.8	11.7	10.6	13.7	1.32	1.28	1.61	1.12	2.32	-	-	-
48		12.0	11.9	11.7	10.6	13.6	1.48	1.38	1.50	1.07	2.17	75.30	13.77	10.93
60		12.2	12.0	11.6	10.7	13.7	1.59	1.46	1.44	1.08	2.53			
72		12.4	12.2	11.7	-	14.0	1.61	1.54	1.39	-	2.85			

Day	6.2	L1	L2	L3	L4	L5	W1	W2	W3	W4	W5
1		11.1	10.9	11.8	10.9	11.5	1.53	1.65	2.25	1.69	1.83
12		11.3	11.0	12.5	11.6	11.9	1.52	1.61	2.42	1.85	1.95
24		11.2	10.7	12.9	11.5	11.9	1.37	1.75	2.53	1.75	1.84
36		11.2	-	13.3	11.5	11.9	1.50	-	2.69	1.71	1.71
48		-	-	13.6	-	11.8	-	-	2.88	-	2.25
60		-	-	14.0	-	-	-	-	3.06	-	-
72		-	-	-	-	-	-	-	-	-	-